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MECHANISMS OF DNA REPLICATION

1. INITIATION POINTS FOR CELLULAR DEOXYRIBONUCLEIC ACID REPLICATION IN HUMAN LYMPHOID CELLS CONVERTED BY EPSTEIN-BARR VIRUS.

Oppenheim A, Shlomai Z, Ben-Bassat H
NCI, Bethesda, MD, 20205
Mol Cell Biol; 1(8):753-762 1981

Replicon size of two Epstein-Barr virus (EBV)-negative lymphoma cell lines, BJAB and Ramos, was measured, as in each of these lines converted by two strains of EBV, derived from the producer cell lines B95-8 and P3HR-1. Logarithmic cultures of randomly growing cells were labeled for 8 min with high-specific activity [3H]thymidine, the cells were gently lysed, and the DNA was spread on microscope slides and autoradiographed. In this manner, replication forks can be observed along the DNA fiber that became labeled during the radioactive pulse. Av replicon length was measured. Significantly shorter replicons (41, 21, and 54% shorter, respectively) were found in three of the four EBV-converted cell lines, BJAB/B95 -8, Ra/B95-8, and Ra/HRIK, indicating that these

HE 20.3122/2

cells had more initiation points than their EBV-negative parents. Replicons that were only slightly shorter than those of the parents were found in BJAB/HRIK cells, although extensive track fusion occurred during the labeling of BJAB/HRIK. This implies that the true av replicon size of these cells was shorter than the observed value. It is suggested that EBV activates new initiation points for cellular DNA replication in EBV-transformed cells. (27 Refs)

EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

2. EPIDERMAL GROWTH FACTOR INITIATES DNA SYNTHESIS AFTER A TIME-DEPENDENT SEQUENCE OF REGULATORY EVENTS IN SWISS 3T3 CELLS: INTERACTIONS WITH HORMONES AND GROWTH FACTORS.

Otto AM, Ulrich MO, Jimenez de Asua L
Friedrich Miescher-Inst., CH-4002, Basel, Switzerland
J Cell Physiol; 108(2):145-153 1981

Swiss mouse 3T3 cells in confluent and quiescent cultures were treated with epidermal growth factor (EGF) in various single and split doses and in combinations with insulin, hydrocortisone, prostaglandin E1 (PGE1), and prostaglandin F2 (PGF2). DNA synthesis was assayed using radioactive labeling. EGF stimulated the synthesis of DNA in quiescent cultures after a lag period of about 15 hr. The final rate of initiation of synthesis followed first-order kinetics and was quantified by a rate constant k. The value of k was affected by administrations of EGF during the prereplicative period. The minimum length of the lag phase was set by the first dose of EGF, independent of concentration, while k was dependent on concentration. Insulin alone did not stimulate DNA synthesis. In combination with EGF it showed a synergistic effect. Insulin did not effect the length of the lag phase. Hydrocortisone, when added within 8 hr of the addition of EGF or EGF plus insulin, inhibited the stimulatory effects of those substances. PGE1 inhibited DNA synthesis by increasing the lag phase. PGF2 had a synergistic effect in combination with EGF. The results suggest that a growth factor first induces a sequence of regulatory events leading to the initiation of DNA synthesis. Then it acts separately to regulate the rate of DNA synthesis. Prostaglandin and EGF apparently act on different regulatory events. (29 Refs)

3. ANDROGEN-INDUCED REPLICATION OF PROSTATE CHROMATIN DNA ISOLATION AND ELECTRON MICROSCOPIC VISUALIZATION OF REPLICATIVE INTERMEDIATES.

Majumdar CA, Anderson KM
Bethesda Res. Lab., Inc., Rockville, MD
Biochim Biophys Acta; 655(1):61-70 1981

A procedure originally developed to isolate transcriptionally active from less active rat ventral prostate chromatin, employing minimal shear and centrifugation through a dense sucrose gradient, was used to separate prostate chromatin actively synthesizing DNA from total chromatin. It was verifi ed that max DNA synthesis in ventral prostates of rats 6 days after castration occurred after administration of testosterone propionate daily for 3 days. When minced ventral prostates from such animals were incubated with [3H]thymidine, and the 'heavy' and 'light' chromatin fractions were separated by sucrose gradient centrifugation, most radioactive DNA was present in the 'light' fraction at the top of the gradient. Incorporation was due to DNA synthesis and not to repair, as judged by inhibition with N-ethylmaleimide. Results of in vitro and in vivo thymidine pulse-chase experiments were consistent with initial labeling of DNA in a replication complex and subsequent sequestration or radioactive DNA in forms resistant to release by the preparative procedure. Although about half the estimated total endogenous DNA polymerase activity detected in vitro was present in the heavy fraction, the apparent specific activity of the enzyme in the 'light' fraction was 5x as high. Lastly, when equal concentrations of DNA from the separated chromatin fractions were shadowed with platinum-palladium and examined by electron microscopy, 5x as many Y-shaped structures were seen in the light fraction. This procedure facilitates the isolation of enzymatically active DNA structures undergoing semiconservative replication and study of their subsequent molecular 'processing' into forms no longer susceptible to separation by this comparatively gentle method of chromatin preparation. Since the method also yields transcriptionally active chromatin fractions from rat liver and Chinese hamster ovary cell nuclei, it should be applicable to the study of DNA synthesis in these and many other cells. (Author abstract) (21 Refs)

4. GUANINE NUCLEOTIDE DEPLETION AND TOXICITY IN MOUSE T LYMPHOMA (S-49) CELLS.

Cohen MB, Maybaum J, Sadee W
(c/o Sadee) Dept. Pharmacy, Sch. Pharmacy, Univ. California, San Francisco, CA, 94143
J Biol Chem; 256(16):8713-8717 1981

Incubation of mouse T lymphoma (S-49) cells with the inosinate dehydrogenase inhibitor mycophenolic acid produced a depletion of both GTP and dGTP, and resulted in growth inhibition, partial reduction in RNA synthesis, and drastic inhibition of DNA synthesis. Similar results suggested to others that the depletion of dGTP is primarily responsible for toxicity. However, guanosine was as effective as deoxyguanosine at preventing mycophenolic acid toxicity although deoxyguanosine was more effective at elevating dGTP levels. Moreover, in hypoxanthine-guanine phosphoribosyl-transferase-deficient mutants of S-49 (6MPR-3-3) deoxyguanosine was unable to prevent mycophenolic acid toxicity or to re-establish normal DNA synthesis, although it returned cellular dGTP but not GTP levels to normal. No other nucleotide levels changed in a way which could account for the toxicity. Incubation of cells with a combination of deoxyadenosine, deoxycytidine, and erythro-9-(2-hydroxy-3-nonyl)adenine produced a selective depletion of dGTP to levels similar to that produced by mycophenolic acid, but did not affect cell growth. Studies with cells synchronized by centrifugal elutriation show that the toxicity of mycophenolic acid is specific to the S-phase of the cell cycle. Addition of actinomycin D at a concentration that inhibited RNA synthesis increased the availability of GTP and re-established normal DNA synthesis in mycophenolic acid-treated S-49 cells. These results suggest that the depletion of GTP rather than that of dGTP produces toxic effects in S-49 cells and that GTP is required for DNA synthesis. (Author abstract) (19 Refs)

5. THE APPARENT INHIBITION OF UROTHELIAL DNA SYNTHESIS IN NEONATAL RATS BY DIETARY SACCHARIN.

Lawson T, Hertzog P

Eppley Inst. Res. in Cancer, Univ. Nebraska Medical Center, 42nd and Dewey Ave., Omaha, NE, 68105

Cancer Lett; 13(3):259-264 1981

The effect of sodium saccharin on urothelial cells was studied in Sprague-Dawley male and female rats. Parent rats were fed a diet containing 1%, 3%, 5%, and 7.5% saccharin. The females received the same diet during pregnancy and up to 21 days after birth. Significant differences in the body wt of the F1 males were only observed after 16 and 21 days in the group that derived from 7.5% saccharin-fed parents. Urothelial DNA synthesis was determined by the labeling index. The peak of DNA synthesis usually occurs between Day 0 and 2 after birth. In animals that derived from saccharin-fed parents the peak was decreased and delayed. Grain counts served as an indicator of 3H-thymidine incorporation into urothelial DNA. In the controls and the 1% saccharin-fed rats more than 80% of the cells showed greater than 11 grains. In the rats that derived from 3, 5, and 7.5% saccharin-fed parents 13.4%, 30.8%, and 14.4% urothelial cells contained greater than 11 grains, respectively. There is no explanation available for the different reaction of the 5%-saccharin group. In F0 rats, saccharin appears to be a non-genotoxic carcinogen and was excreted in the same amount as administered. In the present study the F1 rats showed saccharin-induced cellular changes which could be involved in carcinogenesis. (20 Refs)

6. CHEMICAL ANALYSIS OF GLYCOSAMINOGLYCANS INHIBITING DNA SYNTHESIS.

Aoi Y, Yokota M, Kato I, Hosokawa T

Inst. Medical Science, Univ. Tokyo, P.O. Takanawa, Tokyo 108, Japan

J Med; 12(2/3):127-146 1981

Sulfated glycosaminoglycans having inhibitory activity in cellular and subcellular systems were found in some human tumor tissues. These glycosaminoglycans inhibited DNA synthesis of virus transformed cells (SV40-WIRL-3 cells) more efficiently than DNA synthesis of their parent normal cells (WIRL-3 cells). Sulfated glycosaminoglycans found in normal human and non-tumor tissues did not have as h igh an inhibitory activity on DNA synthesis by cells used in this investigation as those from some human tumor tissues. The former did not inhibit DNA synthesis by virus transformed cells as effectively as DNA synthesis by their normal parent cells. The monosaccharide composition of these sulfated glycosaminoglycans showed N-acetyl glucosamine (Glu-NAc) as a main monosaccharide, and xylose (Xyl), glucose (Glu), galactose (Gal), and hyaluronic acid (Hu-A) as minor monosaccharides. N-acetyl galactosamine was not detected. (Author abstract) (16 Refs)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

7. DNA POLYMERASE ALPHA AND BETA ACTIVITY IN GAMMA-IRRADIATED HELA S3 CELLS.

Leadon SA, Ward JF

Dept. Carcinogenesis, Swiss Inst. Experimental Cancer Res., CH-1066 Epalinges s/Lausanne, Switzerland

Int J Radiat Biol; 40(2):195-204 1981

The acute effects (less than 2 hr) of gamma-irradiation on DNA polymerase alpha and polymerase beta activity in HeLa S3 cells were studied. The enzyme activities were measured in sonicates of the irradiated cells, using an exogenous DNA as template. Both DNA alpha- and beta-polymerase activities decreased following irradiation of the cells. Doses as low as 100 rad significantly reduced the activities of the enzymes. While the activities of both DNA polymerases decreased as the dose received by the cells increased, the major reduction in enzyme activity occurred with doses of 100-200 rad. The reduction in DNA alpha- and beta-polymerase activities was max by 30 min post-irradiation and recovered to control values by 2 hr post-irradiation. (Author abstract) (28 Refs)

8. INHIBITION OF DNA POLYMERASE ALPHA BY BLEOMYCIN.

FitzGerald G, Wick MM

Div. Medicine, Sidney Farber Cancer Inst., Harvard Medical Sch., Dept. Dermatology, Boston, MA, 02115

Biochem Biophys Res Commun; 101(3):734-739 1981

The actions of bleomycin (BLM) on synthetic single (poly[dA]-oligo-[dT]) and double (poly[dA-dT]) stranded templates and on DNA polymerase alpha were examined. The reducing agent DTE enhanced the inhibitory activity of BLM. Only in its presence did BLM have a limited effect on the replication of the single stranded template, assessed via DNA polymerase activity. A plateau of approx 35% inhibition occurred between 10 and 10,000 uM BLM. BLM inhibited double strand replication with or without DTE. In order to determine the site of action of BLM, preincubation of the enzyme or template was followed by physically removing the drug by dialysis. DNA polymerase and single and double stranded templates were separately preincubated in the presence of 2 milliM DTE with or without 0.1 milliM BLM. BLM irreversibly damaged the double stranded template (88% inhibition of replication). BLM had no effect on the single strand ed template. Following removal of BLM from DNA polymerase, a 34% loss of enzyme activity was observed. It was suggested that the 35% inhibition of single stranded replication in the BLM dose response test resulted not from an effect on the homopolymer template but from an alteration of the DNA polymerase enzyme. A Hill plot indicated inhibition of the enzyme alone. BLM was found to have a limited irreversible effect on DNA polymerase alpha attributable to residual activity of the enzyme-inhibitor complex. (14 Refs)

9. HERPES SIMPLEX VIRUS TYPE I DNA POLYMERASE. KINETIC PROPERTIES OF THE ASSOCIATED 3'-5' EXONUCLEASE ACTIVITY AND ITS ROLE IN ARAAMP INCORPORATION.

Derse D, Cheng YC
Dept. Pharmacology and Medicine, Sch. Medicine, Univ. North Carolina at Chapel Hill, Chapel Hill, NC, 27514
J Biol Chem; 256(16):8525-8530 1981

An exonuclease activity copurified with herpes simplex virus type I (HSV-1) DNA polymerase through DNA-cellulose column chromatography and comigrated with DNA polymerase activity on nondenaturing gel electrophoresis at varied polyacrylamide concentrations. A gapped duplex DNA was the preferred substrate for this exonuclease activity since the hydrolytic activity on this type of DNA was much greater than the hydrolysis of either native or heat-denatured DNA. Using 3'-terminally labeled activated calf thymus DNA as substrate, the exonuclease activity was found to be activated by salt and spermidine in a manner identical with HSV-1 DNA polymerase. This activation was accompanied by increases in apparent Km and Vmax values of the activated DNA substrate. Phosphonoformic acid inhibited both DNA polymerase and exonuclease activities uncompetitively with respect to activated DNA and had a Ki of 2.4 uM at an ionic strength of 0.25 u. Of the nucleoside 5'-monophosphates tested only the purine ribonucleotides inhibited the exonuclease activity. The inhibition was noncompetitive with respect to DNA, and GMP was about twice as potent as AMP or IMP. 9-beta-D-arabinosyladenine 5'-monophosphate (araAMP) could be incorporated into DNA by HSV-1 DNA polymerase; however, 9-beta-D-arabinosyladenine 5'-triphosphate would not replace dATP in supporting in vitro HSV-1 DNA synthesis. AraAMP incorporated into primer termini caused a significant decrease in the rate of subsequent primer elongation. These 3'-terminal araAMP residues could be removed by the HSV-1 DNA polymerase-associated exonuclease activity in a manner dependent on GMP concentration. (Author abstract) (21 Refs)

MECHANISMS OF DNA REPAIR

10. SENSITIVITY OF EXCISION REPAIR IN NORMAL HUMAN, XERODERMA PIGMENTOSUM VARIANT AND COCKAYNE'S SYNDROME FIBROBLASTS TO INHIBITION BY CYTOSINE ARABINOSIDE.

Cleaver JE
Lab. Radiobiology, Univ. California, San Francisco, San Francisco, CA, 94143
J Cell Physiol; 108(2):163-173 1981

The effects of cell type, cell cycle stage, deoxycytidine pool sizes, concentration of 1-beta-D-arabinofuranosylcytosine (ara-C), and the structure of the aborted repair patch on inhibition of excision repair by ara-C were studied. Primary human cells were obtained by skin biopsy from normal, xeroderma pigmentosum (XP) variant, and Cockayne's syndrome humans. Parental DNA was labeled by growth in 14C-thymidine for at least two to three generations. S-phase cells were labeled with 3H-thymidine. Cultures were then exposed to UV, 13 joules/square meter, with a 5 hr incubation time. With or without hydroxyurea (HU), ara-C inhibited repair of UV damage and resulted in the accumulation of single-strand breaks in DNA during the G1 and G2 phases. The S-phase was resistant to ara-C inhibition, apparently because of either the presence of a pool of deoxycytidine nucleotides during S-phase or the changing roles of different DNA polymerases. Normal and Cockayne's syndrome cells showed similar numbers of breaks, with XP variant cells slightly higher. The number of breaks increased with increasing concentrations of ara-C. HU, when added to ara-C, extended the inhibition into the S-phase. The results suggest that repair proceeds so that both the damaged segment and the repair patch remain attached to the larger DNA strand. Digestion of DNA with exonuclease III indicated that the size of the patch is about 100 nucleotides. (48 Refs)

11. FLUORESCENT-LIGHT-INDUCED LETHALITY AND DNA REPAIR IN NORMAL AND XERODERMA PIGMENTOSUM FIBROBLASTS.

Ritter MA, Williams JR
Dept. Radiation Therapy, Hosp. Univ. Pennsylvania, 3400 Spruce St., Box 522, Philadelphia, PA, 19104
Biochim Biophys Acta; 655(1):18-25 1981

Cell survival and induction of endonuclease-sensitive sites in DNA were measured in human fibroblast cells exposed to fluorescent light or germicidal UV light. Cells from a xeroderma pigmentosum patient were hypersensitive to cell killing by fluorescent light, although less so than for germicidal UV light. Xeroderma pigmentosum cells were deficient in the removal of fluorescent light-induced endonuclease sites that are probably pyrimidine dimers, and both the xeroderma pigmentosum and normal cells removed these sites with kinetics indistinguishable from those for UV light-induced sites. A comparison of fluorescent with UV light data demonstrates that there are markedly fewer pyrimidine dimers per lethal event for fluorescent than for UV light, suggesting a major role for non-dimer damage in fluorescent light lethality. (Author abstract) (40 Refs)

12. CYTOLOGICAL EVIDENCE FOR DNA CHAIN ELONGATION AFTER UV IRRADIATION IN THE S PHASE.

Minka DF, Nath J
Dept. Medical Genetics, Sch. Medicine, Indiana Univ., Indianapolis, IN
Biochem Genet; 19(3/4):199-210 1981

Human xeroderma pigmentosum cells of the XP12BE (XP classic) excision deficient and XP4BE (XP variant) excision proficient cell lines were studied. Cells were synchronized and 1 mM caffeine added. Six sets of plates were irradiated with UV at 0, 1, 2, 3, 4, and 5 hr during S phase. Six hr after the start of S phase, caffeine was removed from the medium. One plate from each set was labeled with 3H-thymidine (3H-TdR) at 0, 0.5, and 1.5 hr after caffeine removal. Controls consisted of untreated cultures and cultures treated with caffeine or UV. XP classic cells with caffeine had greater 3H-TdR incorporation than cells without caffeine when irradiated at the beginning of S phase. Incorporation of the label declined to non-caffeine levels at the end of S phase. Caffeine did not affect labeling in XP variant cells. Human cells irradiated with UV synthesize lower mol wt DNA than unirradiated cells, due to gaps in the DNA. Repair mechanisms eliminate these gaps within a few hr after irradiation. Results of this experiment indicate occurrence of unscheduled DNA synthesis, possibly chain elongation, during the G2 phase in excision deficient cultures. (30 Refs)

13. CELL CYCLE REGULATION OF DNA REPAIR IN NORMAL AND REPAIR DEFICIENT HUMAN CELLS.

Gupta PK, Sirover MA

Dept. Pharmacology, Temple Univ. Sch. Medicine, 3420 North Broad St., Philadelphia, PA, 19140

Chem Biol Interact; 36(1):19-31 1981

The regulation of nucleotide excision repair and base repair by normal and repair deficient human cells was determined. Synchronous cultures of WI-38 normal diploid fibroblasts and Xeroderma pigmentosum fibroblasts (complementation group D) (XP-D) were used to investigate whether DNA repair pathways were modulated during the cell cycle. Two criteria were used: (1) unscheduled DNA synthesis (UDS) in the presence of hydroxyurea (HU) after exposure to UV light or after exposure to N-acetoxy-acetylaminofluorene (N-AcO-AAF) to quantitate nucleotide excision repair or UDS after exposure to methylmethane sulfonate (MMS) to measure base excision repair; (2) repair replication into parental DNA in the absence of HU after exposure to UV light. Nucleotide excision repair after UV irradiation was induced in WI-38 fibroblasts during cell cycle reaching a max in cultures exposed 14-15 hr after cell stimulation. Similar results were observed after exposure to N-AcO-AAF. DNA repair was increased 2-4 fold after UV exposure and was increased 3-fold after N-AcO-AAF exposure. In either instance nucleotide excision repair was sequentially stimulated prior to the enhancement of base excision repair which was stimulated prior to the induction of DNA replication. In contrast XP-D failed to induce nucleotide excision repair after UV irradiation at any interval in the cell cycle. However, base excision repair and DNA replication were stimulated comparable to that enhancement observed in WI-38 cells. The distinctive induction of nucleotide excision repair and base excision repair prior to the onset of DNA replication suggests that separate DNA repair complexes may be formed during the eucaryotic cell cycle. (Author abstract) (31 Refs)

14. INCORPORATION OF 3H-THYMIDINE INTO THE DNA OF CONTINUOUSLY IRRADIATED LS/BL CELLS.

Juraskova V, Benes L

Inst. Biophysics, Czechoslovak Acad. Sciences, Kralovopolska 135, CS-612 65 Brno, Czechoslovakia

Stud Biophys; 83(1):1-10 1981

The rate of synthesis of DNA in continuously irradiated cells was explored. Mouse lymphosarcoma cells growing in an ascitic form ip were transferred weekly into mice (12-14 wk-old C57BL mice weighing about 25 g). Groups of animals were irradiated continuously at 1 and 5 R/hr. The acute irradiation (2,500-20,000 R) was performed in vitro on cells removed from mice. The actual rate of 3H-thymidine incorporation (3HTI) performed in vitro was increased in continuously irradiated animals compared to normal controls, the number of labeled cells in the S-phase was 20% lower compared with controls. The rate of 3HTI after acute irradiation of continuously irradiated animals was higher than in cells subjected to acute irradiation only. The rate of 3HTI is decreased in relation to the degree of exposure and it is the same in continuously irradiated and unirradiated cells. Hydroxyurea (10 mM), when added to the incubation medium, inhibited 3HTI into DNA at approx the same degree in continuously irradiated and control cells. THe authors concluded the unscheduled DNA synthesis is not involved in the increased 3HTI of continuously irradiated cells. (12 Refs)

15. REPAIR OF LESIONS WHICH CAUSE PREMATURE TERMINATION OF TRANSCRIPTION IN CHICKEN EMBRYO CELLS IRRADIATED WITH ULTRAVIOLET LIGHT.

Hackett PB, Varmus HE, BishopJM

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Virology; 112(2):752-756 1981

The dose-response curves for the synthesis of rRNA and avian sarcoma virus mRNA, after irradiation of virus-infected chicken embryo fibroblast cells, have shoulders. We tested the hypothesis that the shoulders were the result of dark repair of transcription-terminating lesions after irradiation by measuring the amounts of rRNA synthesized at various times after the uv dose was applied. Our data support the hypothesis and indicate that the rate of ***repair in transcribed genes*** of chicken cells is about one or two lesions removed per 10(6) base pairs of DNA per min during the first 3 hr after irradiation. (Author abstract) (17 Refs)

16. THE INDUCTION AND REPAIR OF DNA BREAKS IN RAT EPIDERMIS IRRADIATED WITH ELECTRONS.

Burns FJ, Sargent EV

Inst. Environmental Medicine, New York Univ. Medical Center, 550 First Ave., New York, NY, 10016

Radiat Res; 87(1):137-144 1981

In order to study DNA strand breaks in the tissue where it occurs, rather than i n vitro, rat epidermis was used. Male CD rats were given injections of [3H]thymidine to label DNA. They were then irradiated with 0.8 MeV electrons at the rate of 600 rad/min. Epidermal cells were removed. Alkaline treatment and S1 nuclease reaction were used to 'unwind' the DNA. The cells were incubated in the alkaline soln for specified times. The amount of undigestible double-stranded DNA was measured by a liquid scintillation counter. Alkaline lability of DNA was interpreted as due to single-strand breaks in the DNA. The rate of strand-breakage per unit of DNA was estimated by plotting unwinding curves. With radiation dosages between 300 and 2,400 rad, breaks increased in proportion to the dose. When skin received 1,200 rad, the halftime for DNA repair was 21 min. Previous study had shown that the halftime for repair of carcinogenic injury was between 110 and 240 min. Therefore the results of this experiment suggest that single-strand breaks are not a major repairable component of carcinogenic injury. (16 Refs)

17. ONCOGENIC ACTION OF BETA, PROTON, ALPHA AND ELECTRON RADIATION ON THE RAT SKIN.

Burns FJ

Dept. Environmental Medicine, New York Univ., New York, NY

Oncogenic Action of Beta, Proton, Alpha and Electron Radiation on the Rat Skin. Available from National Technical Information Service, Springfield, VA, as COO-3380-33:, 29 pp., 1981.

Rat skin is being utilized as an empirical model for testing dose and time related aspects of the oncogenic action of ionizing radiation, UV light, and polycyclic aromatic hydrocarbons. Molecular lesions in the skin DNA, including strand breaks and thymine dimers, are being measured and compared to tumor induction. The induction and repair kinetics of molecular lesions are being compared to split dose repair. Modifiers and radiosensitizers are being utilized to test specific aspects of a chromosome breakage theory of radiation oncogenesis. (Author abstract)

18. HYPERTHERMIC POTENTIATION OF THE EFFECTS OF A CLINICALLY SIGNIFICANT X-RAY DOSE ON CELL SURVIVAL, DNA DAMAGE, AND DNA REPAIR.

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Div. Radiation Oncology, Univ. Arizona Medical Sch., Tucson, AZ, 85724

Radiat Res; 87(1):109-120 1981

The synergistic effects of combined hyperthermia (43, 45.5 C) followed immediately by clinically significant x-ray doses (3-6 Gray) were studied using mouse leukemia L1210 cells. Cells were treated by hyperthermia and immediately placed in either 22 C or 4 C baths and then irradiated. Cells from the 22 C bath were used to establish initial extent of DNA single-strand breaks. To determine time courses for DNA repair, cells were irradiated at 22 C and placed in a 37 C-CO2 incubator. DNA single-strand breaks were measured by the alkaline elution technique. Hyperthermic treatment at either 43 or 45.5 C significant red uctions in survival of cells. Radiation response showed a threshold response followed by an exponential decrease in survival. Combining hyperthermia with radiation produced lower survival rates than would be expected from combining the effects of each modality separately. This indicates a synergistic effect of the combination. Significantly the synergism factor was greater at 43 C than at 45.5 C. Hyperthermic treatment resulted in inhibition of DNA single-strand break repair. This was because hyperthermia itself caused DNA strand breakage. (17 Refs)

19. EFFECT OF HYPERTHERMIA ON REPAIR OF RADIATION-INDUCED DNA STRAND BREAKS.

Mills MD, Meyn RE

Dept. Physics, Univ. Texas System Cancer Center, M. D. Anderson Hosp. and Tumor Inst., 6723 Bertner Ave., Houston, TX, 77030

Radiat Res; 87(2):314-328 1981

The effect of combined hyperthermia and ionizing radiation on the level of DNA strand breaks was studied in Chinese hamster ovary (CHO) cells. The alkaline elution technique showed that a prior hyperthermic treatment (43-45 C) rendered the cells more sensitive to the production of single strand DNA breaks by x-rays. Hyperthermia also inhibited the rejoining of DNA strand breaks; the degree of inhibition was dependent upon both temperature and duration of the treatment. The enhancement of radiation-induced DNA strand breaks and the inhibition of strand break repair were found to be related to the heat-induced changes in nucleoprotein composition. (27 Refs)

20. RANGE OF RADIOCHEMICAL DAMAGE TO DNA WITH DECAY OF IODINE-125.

Martin RF, Haseltine WA

Biological Res. Unit, Cancer Inst., 481 Little Lonsdale St., Melbourne 3000, Victoria, Australia

Science; 213(4510):896-898 1981

Studies of the length of DNA fragments produced upon decay of iodine-125-labeled deoxycytidine that was located at a single position within a DNA fragment of defined sequence demonstrate that most radiochemical damage occurs within 15 to 20 A of the site of iodine-125 decay. However, DNA strand breakage was detectable up to 70 A from the site of iodine-125 decay. (Author abstract) (12 Refs)

21. DNA DAMAGE BY HALOALKANES IN HUMAN LYMPHOCYTES CULTURED IN VITRO.

Perocco P, Prodi G

Istituto di Cancerologia, Universita di Bologna, via S. Giacomo 14, 1-40100 Bologna, Italy

Cancer Lett; 13(3):213-218 1981

A study is presented of the action of seven haloalkanes as toxic or DNA damaging agents on human lymphocytes. The parameters studied were the scheduled (replicative) and unscheduled (reparative) DNA syntheses seen as tritiated thymidine [(3h)TdR] uptake in a well-known short-term (4 hr) culture system with or without metabolic activation (use of S-9 mix). The toxic effects of the substances were measured by (3H)TdR uptake for the scheduled DNA synthesis (SDS). The effect of the haloalkanes as DNA dama ging agents was studied with the use of (3H)TdR for uptake of unscheduled DNA synthesis (UDS) and was determined by adding 10 mM hydroxyurea to the culture to block (3H)TdR uptake due to SDS. Metabolic activation was achieved by the method of Ames et al. At the end of the culture period (3H)TdR uptake by the lymphocytes for SDS or UDS was determined by a liquid scintillation spectrometer. When the haloalkanes were added to the wells in a concentration of 5 ug/ml, 1,2-dibromoethane (DBE), trichloroethylene (TCE), carbon tetrachloride (CTC), and chloromethyl methyl ether (CMME) exerted the greater toxic effects whereas the uptake of (3H)TdR in the presence of 1,2-dichloroethane (DCE), chloroform (TCM), and dichloromethane (DCM) was similar to that of the controls. In a second study with incubation of the agents with 10 mM hydroxyurea, addition of the activation mixture only markedly increased (3H)TdR uptake in the cases of CMME and DBE. The results indicate the CMME, DBE, TCE and DCE elicit DNA reparative synthesis in human lymphocytes, indicative of their mutagenic power. Low or negative values of UDS were observed for CTC, DCM and TCM. The values on UDS and SDS found were generally not dose-dependent. (14 Refs)

22. THE INFLUENCE OF NITROIMIDAZOL DERIVATIVES AND IRRADIATION ON DNA SYNTHESIS OF L5178Y-CELLS AND HUMAN LYMPHOCYTES.

Magdon E, Schroder E

Robert-Rossle-Institut, Zentralinstitut fur Krebsforschung Akademie der Wissenschaften, Lindenberger Weg 80, DDR-1115 Berlin-Buch, E. Germany

Stud Biophys; 83(2):147-152 1981

The influence of four nitro derivatives of imidazole on semiconservative DNA synthesis was tested in vitro with L5178Y-cells. It was shown that the substances (1.5 uM/ml cell suspension) had no influence on DNA synthesis if the oxygen pressure was normal. Under hypoxic conditions DNA synthesis was 35% decreased by misonidazole; the other derivatives had no effect on DNA synthesis. The influence of the four derivatives on radiation-induced inhibition of DNA synthesis was tested in human lymphocytes. The cells were exposed to 3,000 rads. It was shown that none of the tested substances had an effect on DNA synthesis if the oxygen pressure was normal. For the study of non-semiconservative DNA synthesis, the lymphocytes were exposed to 6,000 rads. The experiments were conducted in the presence of hydroxyurea. As a criterion for the influence of nitroimidazole compounds, 3H-thymidine incorporation was measured. Up to 2 hr after irradiation no effect of the tested substances on DNA synthesis was observed. (12 Refs) (In GER)

23. INHIBITORS OF NUCLEAR ADP-RIBOSYL TRANSFERASE RETARD DNA REPAIR AFTER N-METHYL-N-NITROSO-UREA. FURTHER EVIDENCE FOR THE INVOLVEMENT OF (ADP-RIBOSE)N IN DNA REPAIR.

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FEBS Lett; 131(1):173-177 1981

The rejoining of single-strand breaks in DNA induced by N-methyl-N-nitroso-urea (MNU) was monitored in the presence of transferase inhibitors thymidine and 5-methylnicotinamide using alkaline sucrose gradients. DNA in mouse leukemia L1210 cells was labeled with 3H-thymidine. Fractionated acid-insoluble radioactivity was estimated by scintillation spectrometry. Because the combination of 3 milliM deoxycytidine with 10 milliM thymidine had no adverse effect on cell reproduction, this mixture could be used to examine the effect of thymidine on DNA repair after exposure to 1 milliM MNU for 20 min. In the absence of enzyme inhibitors, extensive strand rejoining had occurred by 60 min. In samples containing thymidine or thymidine + cytidine, very little increase in DNA size was observed. 5-methylnicotinamide at 5 milliM also clearly slowed DNA strand rejoining after MNU damage. Two potent inhibitors of ADP-ribosyl transferase were thus shown to retard rejoining of single-strand breaks induced by MNU; further support is given to the conclusion that (ADP-ribose)n participates in DNA repair. (20 Refs)

24. O6-ALKYLGUANINE AND STRUCTURAL MODIFICATIONS IN LIVER DNA OF DIALKYLNITROSAMINE-TREATED RATS.

Den Engelse L, Philippus EJ, De Brij RJ

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Cancer Lett; 13(3):219-225 1981

Experiments are reported on the induction of single-stranded (ss) regions in liver DNA of dimethylnitrosamine (DMN)- or diethylnitrosamine (DEN)-treated rats and its possible relation to the presence or repair of O6-alkylguanine. The Sprague-Dawley female rats were subjected to partial hepatectomy and liver DNA was labeled with (methyl 3H)thymidine. The carcinogens were labeled with (14C). Rats were treated with 1, 10, 20 or 30 mg/kg ip of DMN; 24 hr later the isolated liver was fractionated on benzoylated-naphtholated-diethylaminoethanol cellulose columns. The amount of DNA eluting in the formamide fraction, which contains ss or partially denatured regions, increased in a dose dependent manner (16.2, 21.6, 32.5 and 45.8%, respectively). After 6 days this DNA was reduced to 9.8%, indicating the the ss and partially denatured regions disappear much more rapidly from rat liver DNA than do those DMN-induced lesions that give rise to more persistent DNA fragmentation in alkaline sucrose gradients. A comparison of the the NaCl- and formamide-eluted DNA, indicated no significant differences were observed in the extents of alkylation at the O6 and 7-position of guanine. The results indicate, that in the present system, neither the presence nor the repair of O6-alkylguanine is related to the formation of single-stranded regions in rat liver DNA. (13 Refs)

25. INDUCTION OF DNA REPAIR SYNTHESIS IN ISOLATED RAT HEPATOCYTES BY 5-DIAZOURACIL AND OTHER DNA DAMAGING COMPOUNDS.

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Cancer Lett; 13(3):187-193 1981

The effect of 5-diazouracil (5-DU) on isolated hepatocytes from male Wistar rats (approx 200 g body wt) was studied. It was shown that 5-DU causes DNA damage in rat hepatocytes. DNA repair synthesis was determined by means of the bromodeoxyuridine-CsCl gradient centrifugation method. 5-DU induces DNA repair at concentrations between 20 umol and 500 umol. Radioactivity incorporation was much higher in the presence of 500 uM 5-DU compared with that of 1 mM methylmethanesulfonate or 50 uM N-methyl-N'-nitro-N-nitrosoguanidine. DNA repair was also observed after cell treatment with styrene oxide, benzo[a]pyrene and dimethylnitrosamine. The mechanism by which 5-DU damages DNA is unclear. It is unlikely, however, that oxygenases are involved in the formation of DNA-binding products because 5-DU also induces DNA repair in V79 hamster cells which do not contain detectable monooxygenase activity. (15 Refs)

26. INDUCTION IN RATS OF LIVER DNA SINGLE-STRAND BREAKS BY THE POLYCHLORINATED BIPHENYL AROCHLOR 1254.

Robbiano L, Pino A

Istituto di Farmacologia dell' Universita di Genova, Genoa, Italy

Boll Soc Ital Biol Sper; 57(4):407-413 1981

Arochlor 1254 (a mixture of polychlorinated biphenyls) induces benign and malignant tumor growth in mice by prolonged administration. Mutagenic and clastogenic studies done so far gave contradictory results. Single doses of Arochlor 1254 (67, 648 or 1,295 mg/kg) given to male albino rats by gastric gavage does max damage in 4-12 hr and almost complete repair is achieved after 48 hr. Daily administration might result in a cumulative effect, because DNA damage is only partially repaired after 24 hr. Arochlor 1254 is 40 times less carcinogenic than diethylnitrosamine and is 110 times less effective in causing DNA damage. (17 Refs)

27. CHEMICAL RADIATION SENSITIZERS AND PROTECTORS: RECENT DEVELOPMENTS.

Kim JH, Chu FCH

No affiliation given

Clin Bull; 11(1):5-11 1981

Findings and prospects with oxic and hypoxic radiosensitizers and with radioprotectors are reviewed. The key radiosensitive site is evidently the the DNA molecule. As oxic radiosensitizers the halogenated pyrimidines, such as 5-chlorodeoxyuridine, 5-bromo-deoxyuridine, and 5-iodo-deoxyuridine, are structural analogues of thymidine. They are incorporated into replicating DNA,resulting in increased sensitivity to irradiation. Clinical applications of the halogenated pyrimidines has been limited, because the sensitivity of normal and tumor tissues is increased equally. Molecular oxygen has the greatest ability of any agent to sensitize cells to radiation. Apparently its interaction with free radicals prevents repair of radiation-induced lesions. Since solid tumor cells are often hypoxic, chemicals that mimic oxygen effects have been tested. These have the advantage of being slowly metabolized. Paranitroacetophenone has sensitizing properties, but also show

toxicologic and pharmacological problems. Among the nitroimidazole compounds, metronidazole (5-nitroimidazole) and misonidazole (2-nitroimidazole) have shown promise. The sensitizing effect of these compounds is due to the nitro group, with cytotoxicity and drug solubility due to the side chain. Aminothiols, such as cysteine and cysteamine have the ability to protect against radiation. The most promising of these compounds is WR-2721. (24 Refs)

28. BROMODEOXYURIDINE PHOTOLYSIS ASSAY AS A POTENTIALLY USEFUL METHOD FOR ASSESSING DNA DAMAGE AND ITS REPAIR INDUCED BY ULTRAVIOLET LIGHT.

Yatani R, Konishi T, Kusano I, Shiraishi T, Noda M, Ohashi S, Suzuki M
Dept. Pathology, Mie Univ. Sch. Medicine, Tsu, Japan
Mie Med J; 30(3):267-272 1981

The bromodeoxyuridine (BrdUrd) photolysis assay was used to assess DNA damage and repair induced by UV light in three melanoma cell lines, xeroderma pigmentosum (XP), normal human fibroblasts, and HeLa S3 cells. The cells were incubated with 3H-thymidine or 14C-deoxythymidine for 24 hr, incubated in nonradioactive medium for 2 hr, then exposed to 20 Jm-2 of a 254 nanometer (nm) UV light. The cells were then incubated for 24 hr in medium containing hydroxyurea and BrdUrd or deoxythymidine. BrdUrd incorporated into DNA during the repair period by exposing the cell suspension to a 313 nm light. Postreplication repair assays were also conducted. In normal cells, a UV-induced lesion in the DNA was necessary before incubation in BrdUrd synthesized DNA to 313 nm radiation and a UV-induced lesion resulted in the incorporation of BrdUrd throughout the 3H-thymidine cellular DNA. In XP cells, the incorporation of BrdUrd into the DNA after UV-irradiation was negligible. The marked decrease in sedimentation rate seen in normal human fibroblasts after UV, BrdUrd treatment, and 313 nm irradiation did not occur in XP cells although slight differences in XP cells subjected to such treatment were seen compared with those not UV-irradiated. Normal cells became highly sensitive to 313 nm light when they were permitted to repair UV-induced lesions in the presence of BrdUrd, while XP cells did not have that sensitivity, presumably because they cannot make the initial strand breaks which necessarily precede the incorporation of BrdUrd. This assay detects excision repair as a cumulative process and estimates the patch size involved in repair replication as well as interpatch distances. (26 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

29. THREE DIFFERENT HUMAN TUMOR CELL LINES CONTAIN DIFFERENT ONCOGENES.

Murray MJ, Shilo BZ, Shih C, Cowing D, Hsu HW, Weinberg RA
Center Cancer Res., Dept. Biology, Massachusetts Inst. Technology, Cambridge, MA, 02139
Cell; 25(2):355-361 1981

We have obtained foci of transformed mouse cells after transfection of human DNA from colon and bladder carcinoma cell lines and a promyelocytic leukemia cell line. These foci can be shown to contain a large number of human DNA sequences by use of highly repetitive human DNA sequence probes. Cell DNA from primary foci can be used in a subsequent cycle of transfection resulting in secondary foci that contain relatively little human DNA. Secondary foci appear to contain only the human sequences proximal to those responsible for the transformed phenotype. A set of characteristic DNA restriction fragments is found in common among secondary foci derived from each tumor cell line DNA. Comparison of the common DNA fragments found in secondary foci derived from three different human tumor cell lines indicates that these three cell lines contain three different transforming genes. (Author abstract) (32 Refs)

30. NUCLEOTIDE SEQUENCE OF THE CHROMOSOMAL GENE FOR HUMAN FIBROBLAST (BETA1) INTERFERON AND OF THE FLANKING REGIONS.

Degrave W, Derynck R, Tavernier J, Haegeman G, Fiers W
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Gene; 14(3):137-143 1981

The nucleotide sequence of the human fibroblast (beta1) interferon chromosomal gene and its flanking regions were determined. The chromosomal EcoRI fragment of plasmid pgHFIF-4, a recombinant derivative of pBR325, contained the genomic beta1 interferon DNA. Plasmid DNA was prepared by lysozyme-detergent lysis and purified by isodensity centrifugation in CsCl gradients in the presence of ethidium bromide. Dephosphorylation, 5'-terminal labeling, and nucleotide sequence analysis of the DNA fragments were performed using 0.5 mm, 5-10% polyacrylamide gels. A preliminary restriction map of the interferon gene-flanking regions was determined and provided a basis for the nucleotide sequence analysis. Chemical degradation showed the sequence to comprise the 836 base pair (bp) segment corresponding to mRNA data, preceded by a 290 bp sequence up to the EcoRI site of the subcloned DNA fragment, and followed by a downstream sequence of approx 190 bp. No intervening sequences were found in the gene. The upstream region contained an abundance of purines. Four homologous segments were discerned: A at position-31, the Hogness-Goldberg box, B at-93 to-85, presumed the transcription recognition region, C at-57 to-51, and D at-114 to-105. A continuous A+T-rich sequence of some 190 bp flanking the gene downstream was established, an area which showed clear but weak homology with the same region in alpha1 interferon. In the upstream sequence, however, significant homologies were found to extend from position-1 up to at least-135, including segments A, C, and D. (31 Refs)

31. ANALYSIS OF SEQUENCES IN HUMAN DNA RELATED TO THE TRANSFORMING GENES OF MURINE TRANSFORMING VIRUSES (MEETING ABSTRACT).

Prakash K, Tronick SR, Eva A, Devare S, Reddy EP, Srinivasan A, Aaronson SA
National Cancer Institute, Bethesda, MD
RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

Using cloned DNA probes specific for the transforming regions of Moloney murine sarcoma virus (M-MSV) and Abelson murine leukemia virus (A-MuLV), we have detected homologous sequences in human DNA. The M-MSV (mos) probe revealed a single 2.5 kbp band in Eco RI-cleaved DNA, whereas two bands (15 and 4 kbp) were detected using the A-MuLU (abl) probe. These viral transforming gene-related sequences were cloned from human DNAs either by standard gene enrichment techniques or by screening cloned human DNA li-

braries. We have performed structural comparisons of viral and normal mouse cellular mos and abl sequences with their human homologues by utilizing restriction enzyme mapping and heteroduplex techniques. The cloned human DNA probes for the mos and abl related sequences and for their respective flanking regions were also utilized in order to study the expression and topography of their related transcripts in human cells. (no Refs)

32. ISOLATION AND CHARACTERIZATION OF THE HUMAN ONC GENE RELATED TO THE TRANSFORMING GENE (V-FES) OF THE SNYDER-THEILEN FELINE SARCOMA VIRUS (MEETING ABSTRACT).

Franchini G, Wong-Staal F, Dalla Favera R, Gallo RC
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD
RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

Feline sarcoma viruses (FeSV) are genetic recombinants between feline leukemia viruses and transformation specific nucleotide sequences (v-fes) derived from normal cat cells. The homologous cellular sequences (c-fes) are conserved among all vertebrate species. Two of the three isolates of FeSV (Snyder-Theilen and Gardner-Arnstein) have apparently acquired the same c-fes sequences and interestingly, an avian sarcoma virus (Fujinami strain) has also derived its onc gene from the homologous gene in chickens. These results suggest that only a limited number of cellular genes will confer transformation potential, and that these genes are important in normal cell growth and/or differentiation. We have obtained a clone containing c-fes sequences from a human DNA library, using recombinant plasmids containing v-fes sequences of the Snyder-Theilen strain of FeSV as a probe. This clone has a 16 Kilobase (Kb) insert which contains appr ox 11 Kb of flanking cellular sequences 5' to the c-fes locus and probably all of the coding sequences. In order to make a more detailed map, a EcoRI-Hind III DNA fragment containing all the c-fes sequences was subcloned into pBR322. The results showed that the human c-fes locus is more complex than the v-fes sequences. The 1.4 Kb of coding sequences span a region of approx 3 Kb, interrupted by at least three introns. The presence of introns has been identified in the c-fes locus in cat DNA and in another human onc gene (c-sis) related to simian sarcoma virus. (no Refs)

33. A ROUS SARCOMA VIRUS PROVIRUS IS FLANKED BY SHORT DIRECT REPEATS OF A CELLULAR DNA SEQUENCE PRESENT IN ONLY ONE COPY PRIOR TO INTEGRATION.

Hughes SH, Mutschler A, Bishop JM, Varmus HE
Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724
Proc Natl Acad Sci USA; 78(7,part2):4299-4303 1981

The Rous sarcoma virus (RSV)-transformed rat cell line RSV-NRK-2 contains a single complete RSV provirus. We have obtained recombinant lambda clones that contain both ends of the RSV provirus and the flanking rat sequences. The provirus is integrated in unique DNA and is present in only one of the two homologous chromosomes. The rat sequences into which the RSV provirus integrated were also cloned from the RSV-NRK-2 cell line. The sequences of the regions involved in the recombination event have been determined and compared. Our data suggest that, compared with the sequence of viral DNA in the large circular form of unintegrated viral DNA, the provirus lacks two base pairs at each end and that the provirus is flanked by a six-base-pair direct repeat of cellular DNA. This six-base-pair repeat was apparently created during the integration event because this sequence was present only once at the integration site before the provirus was inserted. A survey of eight other independent RSV transformed rat cell lines demonstrates that, in agreement with earlier results, the RSV proviruses have entered different segments of rat cell DNA. We have also determined the sequence of a second virus DNA-host cell DNA junction from a second RSV-transformed rat cell line (RSV-NRK-4) and find that there are no obvious similarities between the two integration sites or between the integration sites and the termini of viral DNA. (Author abstract) (32 Refs)

34. CHARACTERIZATION OF MOUSE CELLULAR DEOXYRIBONUCLEIC ACID HOMOLOGOUS TO ABELSON MURINE LEUKEMIA VIRUS-SPECIFIC SEQUENCES.

Dale B, Ozanne B
Dept. Microbiology, Univ. Texas Health Science Center at Dallas, Dallas, TX, 75235
Mol Cell Biol; 1(8):731-742 1981

The nature of BALB/c-derived DNA sequences in the Abelson murine leukemia virus (A-MuLV) genome and their relationship to homologous sequences in mice (endogenous Abelson sequences) was examined. A DNA fragment of A-MuLV linear DNA was cloned into and Escherichia coli plasmid and used as a probe in Southern blot restriction enzyme analysis of high mol wt cellular DNAs prepared from mouse cells and cells of other species. Endogenous Abelson sequences are arranged similarly in all of the mouse strains examined. Other species possess nucleic acid sequences homologous to A-MuLV-specific sequences. The BALB/c-derived sequences as they appear in the virus are markedly different from the arrangement of homologous sequences in the mouse genome. No obvious rearrangement of endogenous Abelson sequences in the DNA of cells representing end-stage differentiation of the B-cell lineage or in thymocytes was observed. A map of normal mouse cellular sequences that are homologous to the A-MuLV probe is presented. (53 Refs)

35. ISOLATION AND CHARACTERIZATION OF THE REGION OF THE CHICKEN CHROMOSOME CONTAINING C-SRC, THE ENDOGENOUS HOMOLOGUE OF THE ROUS SARCOMA VIRUS TRANSFORMING GENE (MEETING ABSTRACT).

Parker RC, Varmus HE, Bishop JM
Department of Microbiology and Immunology, University of California, San Francisco, CA, 94143
RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

The Rous sarcoma virus transforming gene, v-src, is structurally very similar to a normal gene, c-src, found in all vertebrates. The viral and cellular genes not only share nucleic acid homology but encode proteins of approx 60,000 daltons with highly related peptide maps. We have isolated two overlapping clones spanning more than 30,000 bases of DNA from a recombinant DNA library representative of the chicken genome. Both of these clones hybridize to v-src and together contain all of the sequences responsible for the synthesis of pp60c-src. One clone contains greater than 8000 bases 5' of the region that hybridizes to v-src probes; the other clone extends more than 10,000 bases past the 3' end of the coding sequence. Detailed analysis of these clones revealed that c-src, like many eukaryo-

tic genes, contains intervening sequences. The homology between v-src and c-src not only spans the coding regions of these genes, but includes flanking sequences present both 5' and 3' of v-src. The DNA in these clones does not contain any moderately or highly repeated sequences. It does, however, appear to contain at least two genes in addition to c-src. Sequences within the clones hybridize to three distinct species of poly (A) containing RNA. One of these transcripts is approx 4500 bases long. It hybridizes to probes for c-src and v-src. The two additional transcripts are approx 2000 bases long. One of these is detected by the clone containing sequences 5' of c-src, whereas the other is detected by the clone containing sequences 3' of c-src. (no Refs)

36. ACCESSIBILITY OF SOME REGIONS OF CHROMATIN OF LEUKEMIC CHICKEN CELLS TO SINGLE S TRAND NUCLEASE S1.

Leibovitch SA, Guillier M, Harel J
Laboratoire de Biologie Moleculaire et Physiopathologie Cellulaire, Institut Gustave-Roussy, G. R. N. 8 du CNRS, 16 bis, Avenue Paul Vaillant Couturier, 94800 Villejuif, France
Biochem Biophys Res Commun; 101(3):719-726 1981

Single strand nuclease S1 was used to probe the structure of DNA in Avian leukemic cells infected with Avian Myeloblastosis virus (AMV). Acid-soluble DNA fragments released after incubation of purified nuclei with 2000 units/ml (Aspergillus oryza) nuclease S1 appeared after a 5 min lag, with quantities increasing rapidly to approx 2% of total nuclear DNA at 20-30 min and to approx 4% after 4 hr. Control samples incubated without S1 released less than 1% of total DNA after 3 hr. Size distribution of the purified DNA was determined by agarose slab gel electrophoresis. Between 0-5 min of nuclease digestion only high mol wt (greater than 12,000 base pair) DNA fragments were obtained. Thereafter low mol wt segments appeared which could be resolved into at least the four size classes monomer through tetramer. A comparison of the effects of S1 nuclease and DNAase I on integrated AMV provirus sequences was made by hybridization of enzyme-treated nuclei to AMV-3HcDNA. Partial digestion of leukemic cell DNA with S1 nuclease did not significantly modify hybridization from the greater than 80% level found with non-nuclease-treated DNA. With DNAase I, however, hybridization of the virus-specific probe was reduced nearly to the 20% level obtained with normal embryonic chick cell DNA. S1 nuclease and DNAase I appear not to recognize the same sites in active chromatin regions. Furthermore, a limited number of chick leukemic cell DNA sites were susceptible to S1 nuclease. The resulting DNA fragments appearing as multiples of a monomeric unit were similar to fragments produced by micrococcal nuclease digestion. (18 Refs)

37. GENE STRUCTURE OF CELLULAR SRC AND VIRAL SRC OF RSV (MEETING ABSTRACT).

Takeya T, Junghans R, Ju G, Skalka AM, Hanafusa H
The Rockefeller University, New York, NY, 10021
RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

We have characterized the DNA structure of cellular src (c-src) from chicken and viral src (v-src) of both recovered ASV and Rous sarcoma virus (SR-A). Recovered virus is a recombinant between SR-A td virus and c-src. First, we cloned c-src from a chicken library phage stock constructed by Dodgson et al. Two clones were analyzed extensively by electron microscope and restriction endonuclease mapping. One clone (18.1 kb) contained the entire c-src and 5'-flanking sequences, while the other (18.4 kb) contained the entire c-src and 3'-flanking sequences. The size from 5'-end to 3'-end was about 7.6 kb and c ontained 7 introns and 8 exons. Second, we have compared the restriction pattern of the 2.0 M dalton EcoRI DNA fragment from recovered virus number 1441 with that from SR-A. After digestion with either HaeIII, PstI or HinfI, one fragment of 1441 was observed to be 20 base pairs shorter than the equivalent fragment of SR-A. The sequences were largely homologous (over 95%), however, significant difference was detected around the region just upstream from the supposed N-terminus of p60. The difference might be due to the recombination reaction. It appears most likely that recombination between td virus and c-src takes place after c-src is transcribed and processed into its mRNA. (no Refs)

38. CHROMATIN ASSEMBLY. RELATIONSHIP OF CHROMATIN STRUCTURE TO DNA SEQUENCE DURING SIMIAN VIRUS 40 REPLICATION.

Tack LC, Wassarman PM, DePamphilis ML
Dept. Biological Chemistry, Harvard Medical Sch., Boston, MA, 02115
J Biol Chem; 256(16):8821-8828 1981

The accessibility of five specific DNA sequences to six different single site restriction endonucleases was evaluated in replicating and mature simian virus 40 chromosomes isolated by three different methods. Electron microscopic and gel electrophoretic analysis of the DNA digestion products demonstrated that DNA accessibility in chromatin was established within 400 base pairs of replication forks and remained essentially unchanged during production of mature chromosomes and their subsequent re-entry into the replication pool. Saturating amounts of each enzyme reproducibly cut a fraction of the chromosomes, ranging from 13 to 49%. This is consistent with a nearly random phasing of chromatin structure. Examples in which all chromosomes were either cleaved or intact were never observed. Although variation in the accessibility of DNA sites near the origin of replication could be interpreted as preferred phasing in about 25% of the chromosomes, the finding that two isoschizomers, Hpa II and MsP I, did not cut chromosomes to the same extent precludes an unambiguous interpretation of the extents of cleavage of individual restriction enzymes. Since the extent of DNA cleavage observed at each restriction site was essentially indistinguishable in replicating as compared to mature chromosomes, the accessibility of DNA sequences near the origin is not obviously related to replication. Furthermore, the accessibility of DNA sites on one arm of a single replication fork was the same as the homologous sites on the other arm, consistent with a nearly random phasing of chromatin structure on both arms. This suggests that chromatin assembly occurs independently on the two sibling molecules of a single replicating chromosome. (Author abstract) (72 Refs)

OTHER RELATED STUDIES

39. TOTAL SYNTHESIS OF A HUMAN LEUKOCYTE INTERFERON GENE.

Edge MD, Greene AR, Heathcliffe GR, Meacock PA, Schuch W, Scanlon DB, Atkinson T C, Newton CR, Markham AF
ICI Pharmaceuticals Div., Mereside, Alderly Park, Macclesfield, Cheshire SK10 4TG, England
Nature (Lond); 292(5825):756-762 1981

A 514-base pair fragment of double-stranded DNA coding for human interferon-alpha1 (166 amino acid residues), and containing initiation and termination signals plus appropriate restriction enzyme sites for plasmid insertion, has been totally synthesized. The synthesis involved preparation of 66 oligodeoxyribonucleotides, ranging in size from 14 to 21 residues, plus 1 deoxydecanucleotide, by rapid, solid phase procedures, and enzymatic ligation of the oligonucleotides. After ligation of the synthetic gene to a plasmid vector and transformation of Escherichia coli, clones containing the anticipated gene sequence were obtained. (Author abstract) (55 Refs)

40. ASSIGNMENT OF THE CHLORAMPHENICOL RESISTANCE GENE TO MITOCHONDRIAL DEOXYRIBONUCLEIC ACID AND ANALYSIS OF ITS EXPRESSION IN CULTURED HUMAN CELLS.

Wallace DC
Dept. Genetics, Sch. Medicine, Stanford Univ., Stanford, CA, 94305
Mol Cell Biol; 1(8):697-710 1981

Mitochondrial DNA's (mtDNA's) sequence variants detected by restriction endonucleases were used to distinguish between the mtDNA's of human HeLa and HT1080 cell lines. HaeII digestion yielded a 9-kilobase fragment in HT1080, which was replaced by 4.5-, 2.4-, and 2.1-kilobase fragments in HeLa. Distinctive 1.35-and 0.68-kilobase fragments from HeLa were yielded by HaeIII and HhaI. These restriction endonuclease polymorphisms were used as mtDNA markers in HeLa-HT1080 hybrid and hybrid crosses involving the cytoplasmic chloramphenicol (CAP) resistance. Four hybrids and three Hybrids (4 with HeLa CAP resistance and 3 with HT1080 CAP resistance) contained 2-to 10-fold excesses of mtDNA of the CAP-resistant parent. One hybrid had approx equal proportions of the two mtDNA's. One hybrid was discordant. Thus, CAP resistance is encoded in mtDNA and its expression is related to the ratio of CAP-resistant and CAP-sensitive genomes within the cells. (46 Refs)

41. A SELECTIVE EFFECT OF METHYLGLYOXAL-BIS(GUANYLHYDRAZONE) ON MITOCHONDRIAL DNA.

FeuersteinBG
State University of New York, Buffalo, NY
Diss Abstr Int (B); 42(1):165-B 1981

The effect of methylglyoxal-bis(guanylhydrazone) (MGBG) treatment on the synthesis of mitochondrial DNA (mtDNA) has been examined. This was accomplished by pulse labeling the DNA of treated and untreated cells with 3H-thymidine (TdR) and isolating their mtDNA. A selective inhibition of the synthesis of mtDNA in this system is described. Further characterization on the effect of velocity sedimentation confirms the existence of the effect. This is then compared in terms of dose and of treatment time to established effects of MGBG on mitochondrial ultrastructure, cellular polyamines, nuclear DNA synthesis, and ce ll growth. (no Refs)

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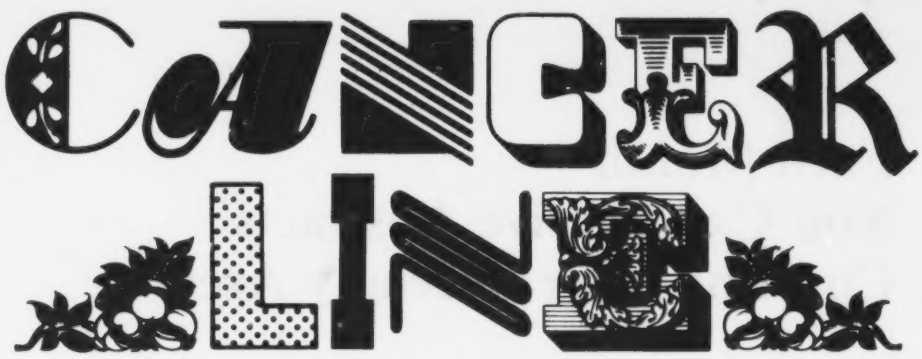

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MECHANISMS OF DNA REPLICATION

1. DNA REPLICATION IN X-IRRADIATED HUMAN LYMPHOCYTES.

Agarwal SS, Katz EJ, Krishan A, Loeb LA
(c/o Loeb) Dept. Pathology SM-30, Univ. Washington Sch. Medicine, Seattle, WA, 98195
Cancer Res; 41(10):3973-3978 1981

Human peripheral blood lymphocytes are well-differentiated cells. Ordinarily, they do not divide and are considered to be in the Go stage of the cell cycle. These cells can be stimulated to undergo DNA replication in culture by mitogens such as phytohemagglutinin. In the present study, we have examined cellular and biochemical events that occur after exposure of lymphocytes to x-irradiation. Irradiation with up to 100 rads,

HE 20.3173/2:

prior to stimulation with phytohemagglutinin, did not interfere with DNA replication. At later periods, DNA replication was inhibited proportional to the amount of radiation. In compar ison to DNA synthesis, the effect of x-irradiation on RNA and protein synthesis in phytohemagglutinin-stimulated lymphocytes was less marked. Furthermore, x-rays did not inhibit either the induction or the continual synthesis of DNA polymerase-alpha or -beta in response to phytohemagglutinin. Kinetic studies with different nucleotide substrates suggest that cellular pools of nucleotides are not significantly altered by x-irradiation. Thus, the inhibition of DNA synthesis in irradiated cells is likely to be due to damage to the cellular DNA template. The inhibition of DNA synthesis was accompanied by accumulation of cells in the G2 and M stages of the cell cycle, suggesting that inhibition of DNA replication by x-irradiation is a postmitotic event. (Author abstract) (38 Refs)

2. MODULATION OF IN VITRO TRANSFORMATION AND THE EARLY AND LATE MODES OF DNA REPLICATION OF UV-IRRADIATED SYRIAN HAMSTER CELLS BY CAFFEINE.

Doniger J, DiPaolo JA
Lab. Biology, NCI, Bethesda, MD, 20205
Radiat Res; 87(3):565-575 1981

The relationship between the effect of caffeine (CF) on post-UV DNA synthesis and its effects on transformation were studied in fresh tertiary Syrian hamster embryo cells (HEC). When HEC treated with 3 joules/square meter radiation were incubated in the presence of CF (0.25 milliM) for 48 hr, the UV-induced transformation frequency was 2-5-fold higher than that obtained with UV irradiation alone. The degree of enhancement by CF was dependent on the interval between UV irradiation and CF addition. Thirty min after HEC were UV-irradiated, the pulse-labeled DNA nascent strands were much smaller than the nonirradiated controls. The addition of CF immediately after UV irradiation had little or no effect on the size of nascent strands during the first 1.5 hr. However, the recovery of size of the nascent strands observed 5-7 hr post-UV was inhibited by CF. Thus, CF inhibited the occurrence of the late mode of replication. During the early mode of replication CF inhibited the rate of elongation of nascent daughter strands in UV-irradiated HEC. CF inhibited the enhancement of chain elongation associated with pretreatment by low UV dose, but had no effect on the size distribution of nascent strands in cells that were pulse-labeled at 0.5 hr post-UV but not chased. CF was also able to convert the late-mode replication back to the early mode. (19 Refs)

3. ARE PYRIMIDINE DIMERS NON-INSTRUCTIVE LESIONS?

Lawrence CW
Dept. Radiation Biology and Biophysics, Univ. Rochester Medical Center, Rochester, NY, 14642
MGG; 182(3):511-513 1981

Published data from yeast and E coli show that base substitution induced by UV in pyrimidine-pyrimidine sequences is not random, and suggest that fidelity of DNA replication is not entirely lost during transdimer synthesis. These observations question whether cyclobutane pyrimidine dimers are truly non-instructive lesions. (Author abstract) (17 Refs)

EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

4. DNA SYNTHESIS IN THYMIC-ACUTE LYMPHOBLASTIC LEUKAEMIA.

Piga A, Ganeshaguru K, Lee N, Breatnach F, Prentice HG, Hoffbrand AV
(c/o Hoffbrand) Dept. Haematology, Royal Free Hosp., Pond St., Hampstead, London NW3 2QG, England
Br J Haematol; 48(4):585-594 1981

Deoxyribonucleoside triphosphate (dNTP) concentrations were measured in bone marrow and peripheral WBC from seven patients with acute Thy-lymphoblastic leukemia (Thy-ALL), 12 patients with acute myeloblastic leukemia and 15 patients with acute non-T, non-B lymphoblastic leukemia (c-ALL), and in thymocytes from patients with myasthenia gravis. Labeled thymidine and deoxycytidine incorporation into DNA was also studied. In Thy-ALL, dNTP concentrations were markedly increased compared with those in the other acute leukemias. The dNTP concentrations in thymocytes were, however, similar to those in Thy-ALL. 3H-nucleoside incorporation studies showed a marked difference in labeled deoxycytidine incorporation and particularly in the deoxycytidine/thymidine DNA labeling ratio between Thy-ALL and the other cell types. We conclude that the pathways of DNA synthesis in Thy-ALL blasts are different from those in the cells from other acute leukemias and some but not all these differences may correspond to differences between normal cortical thymocytes and bone marrow cells. (Author abstract) (37 Refs)

5. DRUG RESISTANCE IN MULTIPLE MYELOMA ASSOCIATED WITH HIGH IN VITRO INCORPORATION OF 3H-THYMIDINE.

Hofmann V, Salmon SE, Durie BG
(c/o Durie) Section Hematology and Oncology, Dept. Internal Medicine, Coll. Medicine, Univ. Arizona Health Sciences Center, Tuscon, AZ, 85724
Blood; 58(3):471-476 1981

In multiple myeloma, tumor cell mass and labeling index correlate with subsequent survival duration, but do not predict for response to treatment. In the present study we have autoradiographically measured the incorporation of 3H-thymidine as determined by the number of grains over the myeloma nuclei in bone marrow aspiration samples. In 33/37 patients with less than 50% tumor regression or progressive disease, the pretreatment grain count was greater than or equal to 20/myeloma nucleus. Conversely, values of less than 20 were found in 27/29 patients who had greater than or equal to 50% cell mass reduction. Survival duration was significantly better (p less than 0.001) in patients with grain counts less than 20. Sixty percent of the patients with both a low labeling index (less than or equal to 3%) and grain count (less than 20) were alive at 48 mo, whereas 15/17 patients with a high labeling index and grain count had a median survival of less than 6 mo. In a subset of 22 patients, there was a significant correlation betwe en in vitro resistance to melphalan, adriamycin, and vincristine as tested in the myeloma stem cell colony assay system and a grain count of greater than 20. We can only speculate as to the reasons for the increased 3H-thymidine uptake by myeloma cells resistant to treatment, however, it could be associated with accumulation of excess DNA and/or increased unscheduled DNA synthesis following injury from alkylating agents. (Author abstract) (26 Refs)

6. STUDIES OF DNA SYNTHESIS AND PLOIDY PATTERN OF HEPATOCYTES IN LIVER CIRRHOSIS AND HEPATOMA BY RADIOAUTOGRAPHY AND MICROSPECTROPHOTOMETRY.

Koike Y, Suzuki Y, Nozawa K, Kamijyo K, Yamamura S, Akahane Y, Kiyosawa K, Nagata A, Huruta S, Nagata T
Second Dept. Internal Medicine, Faculty Medicine, Shinshu Univ., Matsumoto-shi, Nagano Pref. 390, Japan
Kanzo; 22(3):413-421 1981

In order to determine the cell kinetics of hepatocytes in liver cirrhosis and hepatoma, both the DNA synthesis, by means of 3H-thymidine (3H-TdR) radioautography, and the DNA content of hepatocytic nuclei, by means of Feulgen-microspectrophotometry, in cirrhosis and the noncancerous portion of hepatoma with cirrhosis were studied. The incorporation of 3H-TdR into hepatocytes in the noncancerous portion of hepatoma as well as cirrhosis which developed into hepatoma 3 yr later increased markedly. The ploidy classes as measured by microspectrophotometry were widely distributed in the noncancerous portion of hepatoma. Moreover, in the normal liver the DNA content of each nucleus a binucleate cell was almost the same, in contrast with that in paired nuclei the noncancerous portion of hepatoma, which was as different as in hepatoma cells. The ploidy pattern of hepatocytes in patients with liver cirrhosis who had developed hepatoma during follow-up periods of several mo to several yr appears to resemble that in the noncancerous portion of hepatoma. (20 Refs) (In JPN)

7. CYTOTOXIC AND CLASTOGENIC EFFECTS OF SOLUBLE AND INSOLUBLE COMPOUNDS CONTAINING HEXAVALENT AND TRIVALENT CHROMIUM.

Levis AG, Majone F
Inst. Animal Biology, Univ. Padua, Padua, Italy
Br J Cancer; 44(2):219-235 1981

Cr(III) and Cr(VI) compounds of varying solubilities have been tested in vitro for their ability to inhibit cell growth and nucleic acid and protein syntheses in BHK cells, to induce alterations in the mitotic cycle in HEp cells, and to increase the frequency of chromosomal aberrations and sister chromatid exchanges (SCE) in CHO cells. All Cr(VI) compounds, and particularly those containing soluble Cr(VI), such as potassium dichromate and zinc yellow, differentially inhibit macromolecular syntheses in BHK cells, that of DNA being always the most affected. Among Cr(III) compounds, which generally have very low cytotoxicity, chromite is particularly active, and inhibits cell growth and DNA synthesis even more than t he poorly soluble Cr(VI) compounds. Preincubation in growth medium, with or without metabolizing cell cultures, solubilizes considerable amounts of Cr(VI) from zinc yellow and chromite, but significant amounts are also obtained from the most insoluble Cr(VI) pigments. When BHK cells are treated with such preincubated solns, reduction of soluble Cr(VI) to Cr(III) by cell metabolites is seen with all Cr(VI) compounds, accompanied by decreased cytotoxicity. The same differences between Cr(VI) and Cr(III) compounds apply to the cytotoxic effects on mitosis of HEp cells and the clastogenic effects on CHO cells. The activity of chromite, the only Cr(III) pigment capable of significantly increasing the frequency of SCE, is due to contamination with soluble Cr(VI). In contrast to the very low cytotoxicity of Cr(III), much higher chromium levels are detected in the cells incubated with soluble Cr(III) than with the same concentrations of soluble Cr(VI). 50% and 75% of chromium accumulated in the cells during treatments with Cr(VI) and Cr(III) respectively remains firmly bound to the cells, even when they are incubated for up to 48 hr in normal growth medium. Chromium accumulated in the cells after treatment with Cr(III) is most probably bound to the cell membrane, whereas some of the Cr(VI) is transported through the cell membrane and reduced in the cell nucleus. The results of the present investigation are in agreement with those obtained with the same Cr(VI) and Cr(III) compounds in mutagenicity assays in bacteria and carcinogenicity tests in rodents. A re-evaluation of the mechanisms of chromium carcinogenesis is proposed. (Author abstract) (54 Refs)

8. CARBOCYCLIC ANALOGUES OF 5-FLUOROURACIL NUCLEOSIDES.

Shealy YF, Frye JL, DuBois NF, Shaddix SC, Brockman RW
Kettering-Meyer Lab., Southern Res. Inst., Birmingham, AL, 35255
J Med Chem; 24(9):1083-1086 1981

The carbocyclic analogues of 5-fluoro-2'-deoxyuridine (5-FdUrd, 1), 5-fluorouridine, and 5-fluoro-3'-deoxyuridine were prepared by fluorination of the uracil nucleoside analogues with elemental fluorine. The 5-FdUrd analogue (C-5-F-2'-dUrd, 6) was enzymatically phosphorylated to the analogue of 5-FdUrd 5'-phosphate and inhibited the incorproration of 2'-deoxyuridine into DNA of murine colon 26 tumor cells and L-1210 cells in culture. Biochemical studies also indicated that C-5-F-2'-dUrd was a less potent inhibitor of DNA synthesis in tumor cells than was 5-FdUrd. C-5-F-2'-dUrd was cytotoxic (ED50 = 2.5 mcg/mL) to L-1210 cells in culture; the other two analogues were less cytotoxic. C-5-F-2'-dUrd was inactive - or, at best, borderline active - in tests against P-388 leukemia in vivo. (Author abstract) (35 Refs)

9. CLOSE ASSOCIATION OF MOUSE MYELOID LEUKEMIA (M1) CELL DIFFERENTIATION WITH CESSATION OF DNA SYNTHESIS.

Sakagami H, Unten S, Konno K
Dept. Biochemistry, Sch. Medcine, Showa Univ., 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan
Cell Struct Funct; 6(2):147-157 1981

Experiments were carried out to determine whether growth retardation in terminally differentiated cells in culture is due to the artefactual production of dead cells or to some intrinsic phenomenon in the cells. Mouse myeloid leukemia M1 cells that had been induced to differentiate in vitro by 10(-6) M dexamethasone (DM) treatment for varying time periods (0-65 hr) were assayed for DNA synthesis by measuring the incorporation of radioactive thymidine into acid-insoluble material. DNA synthesis began to decline after 10 hr of DM treatment and reached one-half the control level after 65 hr; both DM-treated and control cells incorporated approx 20% of the added thymidine initially added to the culture medium and nearly all of this radioactivity was recovered from the acid-soluble fraction, indicating that there is no significant catabolization and reutilization of incorporated label in nonDNA fractions over the time of the experiment; autoradiographs showed that 96.8-98.6% of the label resided in the nucleus. The administration of greater than 10(-4) M arabinofuranosylcytosine inhibited 95% of the thymidine incorporation. The DM-treated cells were fractionated into nonadherent and adherent subpopulations and the adherent cells were gradient-fractionated into further subpopulations based on their density (that reflects the ratio of nucleus to cell size [N/C ratio]). In general, DNA synthesis declined as cell density declined; autoradiographic analysis revealed that this decrease was due to the accumulation of unlabeled cells (cells that cannot carry out DNA replication)

with a much lower N/C ratio than the labeled cells. When the distribution of DNA content was measured by flow microfluorometry, the DM-treated cell population contained approx 50% fewer S-phase cells than the untreated population. These results indicate that M1 differentiation is closely coupled with the cessation of DNA synthesis. (19 Refs)

10. INHIBITION OF DNA SYNTHESIS IN MOUSE EPIDERMIS AND BREAST EPITHELIUM BY DEHYDROEPIANDROSTERONE AND RELATED STEROIDS.

Pashko LL, Schwartz AG, Abou-Gharbia M, Swern D
Fels Res. Inst., Temple Univ., Philadelphia, PA, 19140
Carcinogenesis; 2(8):717-721 1981

Male ICR mice and female virgin C3H-A/A mice were treated with dehydroepiandrosteronv (DHEA), DHEA-sulfate, DHEA-sulfatide, 16alpha-Br-epiandrosterone (Br-Epi), estradiol-17beta, progesterone, testosterone, or corticosterone. The activity of glucose-6-phosphate dehydrogenase (G6PDH) and the level of incorporation of [3H]thymidine in male mouse epidermis was determined. Incorporation of [3H]thymidine was also measured in female mouse breast epithelium. DHEA and DHEA-sulfatide significantly inhibited G6PDH activity in the epidermis. DHEA-sulfate had no inhibitory effect. Br-Epi was the most active inhibitor of the steroids tested. DHEA and DHEA-sulfatide inhibited the stimulation of [3H]thymidine incorporation by 12-O-tetradecanoylphorbol-13-acetate (TPA) in mouse epidermis. DHEA-sulfate did not have this effect. Br-Epi was much more potent an inhibitor in both the epidermis and breast epithelium than DHEA. Of the sex steroids tested, only corticosterone inhibited TPA-stimulated incorporation of [3H]thymidine. The results indicate that the activity of DHEA, which has been shown to suppress spontaneous breast cancer development in mice, is not limited to breast epithelium. A possible relationship to spontaneous breast cancer in humans is suggested. (25 Refs)

11. A SIMPLE METHOD TO DETERMINE THE MODE OF ACTION OF DRUGS THAT INHIBIT DNA SYNTHESIS.

Fang F, Wu G
Dept. Biochemistry and Molecular Biology, Inst. Basic Medical Sciences, Chinese Academy Medical Sciences, Beijing, People's Republic of China
Yaoxue Xuebao; 16(3):238-240 1981

Many drugs (especially anticancer drugs) exert their action by inhibiting the biosynthesis of DNA. This action can be classified into 2 types: (1) damage to the DNA template, which is usually irreversible; and (2) interference with DNA metabolism, which is usually reversible. Based on the incorporation of tritium-labeled thymidine by Ehrlich ascites carcinoma cells following drug treatment, the mode of action of anticancer drugs could be determined. Thus, decreased thymidine incorporation would suggest a mode of action through DNA template damage while increased thymidine incorporation would mean a mode of action through interference of DNA metabolism. Based on this principle, the anticancer drugs, nitrogen mustard, daunorubicin and zhingguangmycin (bleomycin A5) were found to exert their action on tumor DNA biosynthesis through damage of DNA templates. On the other hand, the inhibitory action of arabinoside cytosine, fluorouracil and methotrexate or DNA biosynthesis was through interference with DNA metabolism. These results agreed well with those determined by other methods. It is concluded that this method is simple and accurate in determining the mode of action of drugs that inhibit DNA biosynthesis. (8 Refs) (In CHI)

12. THE THYMIDINE INCORPORATION INHIBITING SCREENING SYSTEM (TTS) TO TEST CARCINOGENIC SUBSTANCES (A NUCLEAR DNA SYNTHESIS SUPPRESSIVE SHORT TERM TEST).

Amlacher E, Rudolph C
Inst. Pathology, Friedrich Schiller Univ., Ziegelmuhlenweg 1, 6900 Jena, E. Germany
Arch Geschwulstforsch; 51(7):605-610 1981

A short-term screening test is described in which the effects of carcinogens on DNA synthesis were measured by the determination of the mean number of silver grains per nucleus in the renal tubule and liver epithelia of 14-day-old CBA mice following ip injection of carcinogen. Fifteen hr after the injection of 0.1 ml of the dissolved chemical in a concentration representing 15/30% of the LD50, 3H-thymidine was injected ip. Autoradiographs of the nuclei of the renal tubular epithelia and of the liver epithelium were examined by visual silver grain counting. From this test, it was demonstrated that carcinogenic compounds of several different chemical classes (nitrosated amines, azo dyes, azo compounds, aliphatic iso- and heterocycles, aromatic amines, and metallic ions) partially inhibited the nuclear incorporation of thymidine in a significant manner over a time period longer than 24 hr. This decrease was expressed as a decrease of the mean of the silver grain number per nucleus in comparison with unaltered controls. This decrease was used as a criterion for the suppressive effect of carcinogenic substances on the rate of nuclear DNA synthesis. No false-positive results were obtained for noncarcinogenic compounds. The effectiveness of this test is discussed. (6 Refs)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

13. EVIDENCE FOR TWO FORMS OF REVERSE TRANSCRIPTASE IN HUMAN PLACENTA OF A PATIENT WITH BREAST CANCER. PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF THE ENZYMES.

Vogel A, Chandra P
(c/o Chandra) Zentrum der Biologischen Chemie, Abteilung fur Molekularbiologie der Universitat Frankfurt/Main, Theodor-Stern-Kai 7, D-6000 Frankfurt (Main) 70, W. Germany
Biochem J; 197(3):553-563 1981

Two DNA polymerases with properties of viral RNA-directed DNA polymerase were found in the placenta of a patient with breast cancer. Both enzyme activities were purified by column-chromatographic procedures or by preparative isoelectric focusing. The most distinguishing feature of the two enzymes is their specificity to transcribe (rA)n.(dT)12 or (rC)n.(dG)18. The two enzymes differ with respect to their elution profiles from the phosphocellulose column, isoelectric point, mol wt, bivalent-cation requirements and thermal stability. Serological analysis of the (rA)n.(dT)12-activated enzyme showed that this enzyme is immunologicaly not related to DNA polymerase-gamma, or to any of the reverse transcriptases purified from retroviruses of avian, murine and subprimate origin. However, the activity of this enzyme was neutralized by antibodies to reverse transcriptase purified from human spleen of a patient with myelofibrosis. Attempts to purify reverse transcriptase from normal human placenta were repeated-

ly unsuccessful. Once the crude homogenate of normal human placenta was freed from endogenous nucleic acids, no (rC)n.(dG)18-dependent activity could be detected. (Author abstract) (17 Refs)

14. UTILIZATION OF 5-FLUORO-2'-DEOXYURIDINE TRIPHOSPHATE AND 5-FLUORO-2'-DEOXYCYTIDI NE TRIPHOSPHATE IN DNA SYNTHESIS BY DNA POLYMERASE ALPHA AND BETA FROM CALF THYMUS.

Tanaka M, Yoshida S, Saneyoshi M, Yamaguchi T
Hematological Disease Center, Nagoya Natl. Hosp., Sannomaru, Naka-ku, Nagoya, Japan
Cancer Res; 41(10):4132-4135 1981

Chemically synthesized 5-fluoro-2'-deoxyuridine 5'-triphosphate and 5-fluoro-2'-deoxycytidine 5'-triphosphate were used efficiently as substitutes for DNA synthesis catalyzed by DNA polymerases alpha or beta from calf thymus. 5-fluoro-2'-deoxyuridine 5'-triphosphate and 5'-fluoro-2'-deoxycytidine 5'-triphosphate were incorporated into DNA in place of deoxythymidine 5'-triphosphate and deoxycytidine 5'-triphosphate, respectively. The incorporated pyrimidine analogs supported further elongation of DNA. The apparent Km's for 5-fluorodeoxyuridine 5'-triphosphate in the reaction of DNA polymerase alpha and beta were 4.3 and 15.4 uM, while those for 5-fluorodeoxycytidine 5'-triphosphate with DNA polymerases alpha and beta were 7.7 and 8.8 uM, respectively, which are comparable to Km's for natural substrates. These results suggest the new possibility that the fluorinated pyrimidines are incorporated into DNA via their triphosphate forms to exhibit their cytostatic actions. (Author abstract) (18 Refs)

15. RIBONUCLEOTIDE REDUCTASE IN CULTURED MOUSE LYMPHOMA CELLS. CELL CYCLE-DEPENDENT VARIATION IN THE ACTIVITY OF SUBUNIT PROTEIN M2.

Eriksson S, Martin DW
Howard Hughes Medical Inst. Lab., Univ. California, San Francisco, CA, 94143
J Biol Chem; 256(18):9436-9440 1981

Ribonucleotide reductase is responsible for the production of the deoxyribonucleotides required for DNA synthesis. The enzyme is composed of two dissociable subunits, proteins M1 and M2, which are inactive alone, but are fully active when combined. From mouse S49 T lymphoma cells we have isolated and separated the two subunits and used each for determining the activity of the complementary subunit in extracts from cells of different phases in the cell cycle. Treatment of S49 cells with cAMP analogs (eg Bt2cAMP) results in the protein kinase-dependent arrest of the cells in the G1 phase of the cell cycle. Ribonucleotide reductase (holoenzyme) activity fell in S49 cells treated for more than 16 hr with Bt2cAMP but was unchanged during short term treatments. The activity of protein M2 was decreased in parallel to the overall activity of ribonucleotide reductase, while protein M1 activity changed less. Removal of Bt2cAMP after 24 hr exposure resulted in increased holoenzyme and protein M2 activities. Centrifugal elutriation of exponentially growing S49 cells separated cells into a 90% pure G1 cell population, a mixture of G1 and early S phase cells and a 95% pure S phase/G2 cell population. The specific catalytic activity of protein M1 was the same in all these fractions while that of protein M2 was decreased 60% in the G1 cell population. These results demonstrate that the ribonucleotide reduction necessary for DNA synthesis is regulated in a cell cycle-dependent fashion by the activity of the protein M2 subunit of ribonucleotide reductase. (Author abstract) (32 Refs)

16. EVIDENCE FOR GENETICALLY INDEPENDENT ALLOSTERIC REGULATORY DOMAINS OF THE PROTEIN M1 SUBUNIT OF MOUSE RIBONUCLEOTIDE REDUCTASE.

Eriksson S, Gudas LJ, Clift SM, Caras IW, Ullman B, Martin DW
Univ. California, San Francisco, CA, 94143
J Biol Chem; 256(19):10193-10197 1981

Ribonucleotide reductase is responsible for the reduction of the 2'-hydroxy moiety of all four ribonucleoside diphosphates to the corresponding deoxyribonucleotides. The overall activity of the enzyme is regulated by the allosteric effectors ATP (activator) and dATP (inhibitor), and the enzyme's substrate specificity is also controlled by nucleotide effectors. For instance, wild type ribonucleotide reductase from mouse T-lymphoma (S49) cells requires dGTP as a positive effector for ADP reduction. This effect of dGTP causes a reciprocal inhibition of CDP reduction. The dGuo-L mutant cell line, resistant to growth inhibition by exogenous deoxyguanosine, contains a nucleotide-binding subunit, protein M1, that conveys to its CDP reductase an insensitivity to dGTP (and dTTP) inhibition. The dGuo-L protein M1 also shows a decreased capacity to use ADP as a substrate, and therefore, the regulation of the substrate specificity is altered in the mutant protein M1. Another mutant cell line, dGuo-200-1, is resistant to deoxyadenosine and its ribonucleotide reductase is abnormally resistant to inhibition by dATP. The isolated mutant protein M1 from dGuo-200-1 cells has a CDP reductase activity which is stimulated by dATP, unlike the wild type enzyme which is inhibited by dATP. It appears that this mutant enzyme has lost the capacity to distinguish between dATP and ATP, but is still sensitive to regulation by dGTP and dTTP. Thus, the site of protein M1 regulating overall activity is altered in the dGuo-200-1 mutant, while the site regulating substrate specificity is normal. These characteristics of the mutants provide genetic evidence for two independent allosteric domains of protein M1, each responsible for a different aspect of nucleotide sensitivity of ribonucleotide reductase. (Author abstract) (21 Refs)

MECHANISMS OF DNA REPAIR

17. ALKALINE SUCROSE SEDIMENTATION ANALYSIS AS AN INDICATOR OF REPAIR CAPABILITY OF XERODERMA PIGMENTOSUM FIBROBLASTS FOR 4-NITROQUINOLINE-1-OXIDE DAMAGE.

Walker IG
Dept. Biochemistry, Univ. Western Ontario, London, Ontario N6A 5C1, Canada
Carcinogenesis; 2(8):691-695 1981

Fibroblasts were obtained from xeroderma pigmentosum (XP) strains in complementation groups designated A, B, C, E, and Variant, and from normal strains designated Ro, Gr, Fa, and Bu. Cells from all groups were exposed to 1 uM 4-nitroquinoline-1-oxide (4NQO) for 1 hr. They were either analyzed immediately or reincubated to allow repair of DNA damage. DNA sedimentation was measured in an alkaline sucrose gradient. In the brief lysis, gradients were allowed to stand in the dark for 30 min before centrifuging. In the long lysis, gradients were allowed to stand overnight before centrifuging. The long

lysis technique showed repair of DNA in the normal strains and in the XP variant and group E strains. This repair was 50% complete 3 hr after removal of the 4NQO and fully complete after 6 hr. No repair was demonstrated in XP groups A, B, and C. The brief lysis technique showed DNA repair which was completed by 6 hr after removal of 4NQO. The repair was the same for all strains. The long lysis technique demonstrates a type of DNA damage not apparent under brief lysis. The results with the longer technique parallel results from other methods that indicate that cells from groups A, B, and C do not repair this type of damage. When alkaline sucrose gradients are used to measure DNA damage, the use of both the brief and long lysis methods is recommended. (26 Refs)

18. CLASSIFICATION OF CHEMICAL AGENTS AS TO THEIR ABILITY TO INDUCE LONG- OR SHORT-PATCH DNA REPAIR IN HUMAN CELLS.

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Biology Div., Oak Ridge Natl. Lab., Oak Ridge, TN, 37830
Mutat Res; 83(2):159-169 1981

The excision repair response of normal human fibroblasts (NHF) and of xeroderma pigmentosum (XP) cells to 33 chemicals was compared with that to UV irradiation (which is known to induce 'long-patch' repair of about 90 nucleotides) and to gamma irradiation (which is known to induce 'short-patch' repair of 3-4 nucleotides) by the 5-bromodeoxyuridine (BrdU) photolysis assay that quantitates unscheduled DNA synthesis. The following chemicals induced long-patch repair of 30-70 nucleotides in length and may be classified as UV-mimetic agents: N'-acetoxyacetylaminofluorene, ICR-170, 4-nitroquinoline-1-oxide, diarylcarbamate, 7-bromomethylbenzanthracene, dimethylbenzanthracene-5,6-epoxide, 7beta,8alpha-diol-9beta,10beta-epoxybenzo[a]pyrene, 7beta,8alpha-diol-9alpha,10alpha-epoxybenzo[a]pyrene, ethyl methanesulfonate, methyl methanesulfonate, and N-hydroxy-1-naphthylamine. The other chemicals elicited short-patch repair in NHF cells; the measurable DNA repair activity was at the limit of resolution of the sucrose density gradient technique employed to assay it. XP cells could not repair damage by these latter chemicals to the same extent that NHF cells could. It appears that, for the chemicals tested here, the ability of an agent to elicit a long-patch DNA repair response from NHF is a property of the most active direct-acting carcinogens. (23 Refs)

19. APHIDICOLIN: AN INHIBITOR OF DNA REPAIR IN HUMAN FIBROBLASTS.

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Dept. Genetics, Univ. Coll., Singleton Park, Swansea SA2 8PP, Wales
Carcinogenesis; 2(8):795-797 1981

Normal human fibroblasts and excision-deficient xeroderma pigmentosum (XP) fibroblasts were labeled in vitro with [3H]thymidine. They were then left unirradiated or exposed to 254 nanom UV. Cultures were then reincubated with media containing aphidicolin (2.5 ug/ml), an inhibitor of DNA polymerase alpha; with cytosine arabinoside (araC: 10(-4) M), an inhibitor of semi-conservative and repair replication; with both aphidicolin and araC; or neither compound. Analysis of DNA showed that UV-induced DNA single-strand breaks accumulated equally in normal fibroblasts exposed to araC or to aphidicolin. The presence of both compounds in the medium did not alter the results obtained with only one. Neither compound had any effect on the excision-deficient XP cells. The results indicate that DNA polymerase alpha does play a role in the excision repair of DNA. (14 Refs)

20. SISTER CHROMATID EXCHANGE, DNA REPAIR, AND SINGLE-GENE MUTATION.

Carrano AV, Thompson LH
Lawrence Livermore Natl. Lab., Livermore, CA
Proceedings of the ICN-UCLA Symposia on Mechanisms in Chemical Carcinogenesis, held in Keystone, CO, February 22, 1981. Available from National Technical Information Service, Springfield, VA, as UCRL-85849:, 22 pp., 1981.

Sister chromatid exchange (SCE) has been studied in cultured mammalian cells with regard to the nature of the inducing lesion, mutation induction, and factors that modify the observed frequency following mutagen exposure. SCEs can be induced by a wide spectrum of DNA lesions and, for nine agents examined, the frequency of induced SCE is linearly related to induced single-gene mutation. Further, a deficiency in DNA repair may alter the expression of both SCE and mutation in a quantitatively similar manner. The frequency of SCE induced by mitomycin-C is suppressed in heterochromatin relative to euchromatin and, in non-dividing lymphocytes, the lesions leading to the formation of SCEs may persist for several mo. (Author abstract)

21. EFFECT OF INTERFERON ON REPAIR OF DAMAGED DNA.

Sinel'shchikova TA, Zasukhina GD
Inst. General Genetics, Moscow, USSR
Dokl Akad Nauk SSSR; 258(5):1231-1232 1981

The effect of interferon (IF) on the rate of repair of DNA breaks induced by 4-nitroquinoline-1-oxide was studied in cultured chick fibroblasts and in human lymphocytes. Coefficient of repair in IF-treated cells was 52%, compared with 41% in nontreated control cells. (4 Refs) (In RUS)

22. LIGHT-INDUCED CHROMATID DAMAGE IN HUMAN SKIN FIBROBLASTS IN CULTURE IN RELATION TO THEIR NEOPLASTIC POTENTIAL.

Parshad R, Gantt R, Sanford KK, Jones GM, Camalier RF
Dept. Pathology, Coll. Medicine, Howard Univ., Washington, DC, 20059
Int J Cancer; 28(3):335-340 1981

Skin fibroblasts from ataxia telangiectasia and xeroderma pigmentosum (XP) donors and from the XP sib (possible heterozygote), all genetically predisposed to a high risk of cancer, show an increased susceptibility to light-induced chromatid breaks after culture in vitro. Light-induced chromatid breaks were shown previously to result from generation of hydrogen peroxide (H2O2) during light exposure. The level of susceptibility attained is significantly higher than that observed in 13 lines of fibroblasts from normal skin of donors ranging in age from 3 days to 92 yr or from fetal skin tested at various population doubling levels. Two lines of normal skin fibroblasts transformed by chemical carcinogens to neoplastic cells also show a significant increase in susceptibility as compared with their untransformed controls. These data indicate for human cells, as reported earlier for mouse cells, an association between enhanced susceptibility to light-induced chromatid damage and neoplastic potential; this association is further supported by the high susceptibility of cells derived from a human adenocarcinoma. Two observations are consistent with the concept that the increased susceptibility does not result from greater initial damage to the DNA of the neoplastic cells. First, activities of the ubiquitous H2O2 scavenging enzyme, glutathione peroxidase, are similar in the paired normal and neoplastic cell populations. Second, cells of the paired lines are equally

sensitive to DNA breakage by exogenous H2O2. The enhanced susceptibility associated with neoplastic potential may result from an impaired capacity to repair DNA rather than a greater initial sensitivity of the neoplastic cells to the damaging agent. (Author abstract) (35 Refs)

23. A NEW HUMAN PHOTOSENSITIVE SUBJECT WITH A DEFECT IN THE RECOVERY OF DNA SYNTHESIS AFTER ULTRAVIOLET-LIGHT IRRADIATION.

Fujiwara Y, Ichihashi M, Kano Y, Goto K, Shimizu K
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J Invest Dermatol; 77(3):256-263 1981

A sun-sensitive, 8-yr-old male patient (termed UV(8)1KO) with only acute recurrent sunburns and without any other physical or neuromental retardations was studied. The patient's skin exhibited lowered minimal erythema doses between 280 and 300 nm monochromatic wavelengths without delayed peaking of erythema. UV(8)1KO skin fibroblasts in culture were 5-fold more sensitive to 254 nm UV killing than normal cells, though the response of obligatory heterozygotes was normal. UV(8)1KO cells showed defective recovery of DNA synthesis after 254 nm UV irradiation with 1 and 5 J/m2, at which dose the recovery occurred in normal cells. The base line level of sister-chromatid exchanges (SCEs) was higher in UV(8)1KO cells (10-12 SCEs/ce ll) than in normal cells (5 SCEs/cell), although the induction rate of SCEs by 254 nm UV in UV(8)1KO cells was the same as in normal cells. Clinical, cellular and molecular characteristics and comparison to those in the other photodermatoses can make a clear distinction of UV(8)1KO from the others. Thus, the UV(8)1KO disorder is put forward as a new photodermatosis with a defect in the recovery of post-UV DNA synthesis. (Author abstract) (38 Refs)

24. ARE INDUCIBLE COMPONENTS INVOLVED IN THE REPAIR OF IRRADIATED NEURONAL AND BRAIN TUMOUR DNA?

Wheeler KT, Wierowski JV, Ritter P
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Int J Radiat Biol; 40(3):293-296 1981

Repair of irradiated neuronal and brain tumor DNA was studied in male Fisher 344 rats bearing 100-200 mg intracerebral 9L brain tumors irradiated with doses of 12.5 or 25.0 Grays (Gy) of Cesium-137 gamma rays at a dose rate of 9.21 Gy/min. After single doses of either 12.5 or 25.0 Gy, the restoration of chromosomal DNA structure in cerebellar neurons was slower than that in intracerebral tumor cells. DNA repair kinetics after a second dose of 12.5 or 25.0 Gy were identical to those obtained after single dose for both tumor cells and cerebellar neurons. Mean of all values on the plateau region of each curve after a single dose was not statistically different from the mean of values obtained after fractionated doses. Results indicated that the slower DNA repair in permanently nondividing neurons is a fundamental property of these cells and not an artifact of the high dose used initially. Evidence did not suggest that the slower repair results from the need to induce a specific component of the repair system. Consequently, difference in quality, quantity, or type of repair enzymes or difference in accessibility of these enzymes to or interference with their operation at the lesions are more attractive hypotheses to explain the slower DNA repair in neurons as compared with brain tumor cells. (5 Refs)

25. THE RESPONSE OF L5178Y-R AND L5178Y-S CELLS TO X RAYS UNDER OXIC AND HYPOXIC CONDITIONS.

Szumiel I, Budzicka E, Niepokojczycka E, Wlodek D, Beer JZ
Dept. Radiobiology and Health Protection, Inst. Nuclear Res., PL-030195 Warsaw, Poland
Radiat Res; 87(3):592-601 1981

The oxygen effect on two strains of murine lymphoma cells, L5178Y-R and L5178Y-S, which are inversely cross-sensitive to x-rays and UV light, was studied and the phenomena following x-ray exposure under oxic and anoxic conditions were compared. Both cells strains were less sensitive to x-rays under hypoxia; hypoxic conditions affected the survival of both strains to the same degree. Under oxic conditions, exposure to x-rays affected DNA synthesis more markedly in L5178Y-S cells than in L5178Y-R c ells. Under hypoxic conditions, [3H]deoxythymidine incorporation was not affected by irradiation in either of the cell strains. Thus, in the case of L5178Y-S cells, a clear difference could be seen between the incorporation rates at the same survival level under oxic and hypoxic conditions. During incubation of L5178Y-S cells irradiated under oxic conditions, a shift occurred toward DNA contents apparently higher than that characteristic for the control G2 cells. This was particularly evident in the 21-hr sample. The normal distribution was once again observed in the 72-hr sample. Under hypoxic conditions, DNA content distribution was less disturbed by irradiation and remained close to the normal one during the postexposure incubation. The results seem to be compatible with the assumption that the rejoining of single-stranded breaks is slow in L5178Y-S cells. (29 Refs)

26. POSTRADIATION DNA REPAIR IN MAMMALIAN CELLS AFTER COMBINED EXPOSURE TO HYPERTHERMIA, 8-BROMOCAFFEINE, AND ACTINOMYCIN D.

Rezvaia SP, Khanson KP
Central Res. Roentgenoradiological Inst., Leningrad, USSR
Radiobiologiia; 21(3):326-329 1981

The effect of combined exposure to hyperthermia, 8-bromocaffeine (BC), and actinomycin D (AD) on DNA repair was studied in irradiated (5,000 rads) LL cells. The level of double-stranded DNA after incubation with BC or AD at 41 C was significantly greater than that at 37 C. These findings indicate that hyperthermia does not enhance the inhibitory effect of BC and AD. (14 Refs) (In RUS)

27. LETHAL EFFECTS OF NATURAL SOLAR ULTRAVIOLET RADIATION IN REPAIR PROFICIENT AND REPAIR DEFICIENT STRAINS OF ESCHERICHIA COLI: ACTIONS AND INTERACTIONS.

Tyrrell RM, Souza-Neto A
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Photochem Photobiol; 34(3):331-337 1981

The inactivation of repair proficient (Escherichia coli K12 AB 1157, E coli B/r) and repair deficient (E coli K12 AB 1886 uvrA, AB 2463 recA and AB 2480 uvrA recA) strains of bacteria by noon sunlight has been measured. The use of biological dosimetry based on an UV sensitive strain of Bacillus subtilis spores has allowed a quantitative comparison of bacterial inactivation by solar, 254 and 302 nm radiations. Our analysis indicates that: (1) uvrA and recA gene products are involved in repair of a substantial portion of the solar DNA damage, (2) 302 nm is a more appropriate wavelength than 254 nm to rep-

resent the DNA-damaging action of sunlight and that (3) repair proficient strains are inactivated by sunlight more rapidly than expected from the levels of DNA damage induced. When populations of repair proficient bacteria are exposed to noon sunlight for 20 min, they become sensitive to the lethal action of far-UV (254 nm), MMS (0.1 M) and to a lesser extent, mild heat (52 C). (Author abstract) (18 Refs)

28. GENETIC AND MOLECULAR EVENTS IN THE REPAIR OF DNA EXPOSED TO PSORALEN + NEAR-UV LIGHT IN HAEMOPHILUS INFLUENZAE.

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Indian J Exp Biol; 19(2):105-111 1981

The removal of crosslinks from DNA, sedimentation properties of DNA undergoing repair, and DNA synthesis following exposure of Hemophilus influenzae cells to psoralen (P) and near-UV light (NUV) were studied. In most cases, cells were exposed to 1 ug/ml psoralen for 15 min at 37 C, then to NUV (360 nanometers) at a dose rate of 1.2 x 10(2) Joules/square meter/sec at a distance of 25 cm or a dose rate of 5.2 x 10(2) Joules/square meter/sec at a distance of 9 cm from the sample. None of the known recombination or biotransformation-defective strains were highly sensitive to PNUV, while H influenzae wild type strains were 4x more sensitive than Escherichia coli to PNUV inactivation. The rec1 gene does not have a significant role in the repair of PNUV-damaged DNA. Only KW31 and Rd (wild-type) strains were proficient in removing crosslinks; indicating that some other lesion is also important for the lethal action of PNUV. Sedimentation through alkaline sucrose gradients demonstrated that immediately after PNUV exposure cellular DNA sedimented faster than normal. Upon incubation of cells in growth medium, DNA was released from the fasi-sedimenting complex and part of it sedimented at the position of control DNA. One fraction of DNA underwent strand cutting and sedimented slowly. This latter fraction disappeared after longer periods of incubation. All the DNA then sedimented at the position of untreated DNA. In the uvr2 and PS07 strains, release of DNA from fast-sedimenting material was slow. (27 Refs)

29. OXYGEN-EFFECT AS AN INHIBITION OF REPAIR: RADIATION STUDIES ON EXCISION REPAIR DEFICIENT MEI-9L1-EMBRYOS OF DROSOPHILA.

Fritz-Niggli H, Buchi C, Schweizer P
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Radiat Environ Biophys; 19(4):265-274 1981

A repair deficient mutant of Drosophila melanogaster, mei-9L1, was used to investigate the oxygen effect on excision repair. Embryos were irradiated wi h 200 kilovolt peak x-rays in air and in oxygen-free N2 20 before and during irradiation. Fundamental differences between mei-9L1 embryos and normal +/+ embryos included the following. The spontaneous lethality of homozygous mei-9L1 embryos was strongly increased coinciding with an increased chromosome instability of the mutants. The 1.75 and 4 hr embryos of the mutants were more sensitive to radiation than normal embryos. The oxygen effect was considerable in the normal embryos, with oxygen enhancement ratios approach 5 in 1.75 hr embryos, while it was practically negli gible in 4-hr mei-9L1 embryos and was reduced in 1.75-hr embryos. The nature of the damage depended on the dose, the ability to repair, and on the presence of oxygen. The ability to repair was thus shown to be associated with the oxygen effect. The spontaneous lethality and the increase in radiosensitivity in the heterozygous mei-9L1 embryos depended upon the genotype of the mother. (29 Refs)

30. A SIMPLIFIED METHOD FOR MEASURING SCHEDULED AND UNSCHEDULED DNA SYNTHESIS IN HUMAN FIBROBLASTS.

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Toxicology; 21(2):151-158 1981

Experiments were carried out to attempt to develop a simple, rapid method to assay unscheduled DNA synthesis (UDS) that could serve well in large-scale screening studies for carcinogenic activity. Tritium-labeled thymidine incorporation into normal human fibroblasts (HF) and into HF blocked DNA synthesis by hydroxyurea (HU) was measured in the presence of a variety of known carcinogens, precarcinogens, and noncarcinogens. Positive results (a dose-related increase in UDS in the treated inhibited cells greater than 2x that in control inhibited cells) were obtained with most of the carcinogens and negative results with most of the noncarcinogenic compounds. The method compares favorably with other more difficult and time-consuming UDS assays. (15 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

31. 7-METHYLGUANINE IN POLY(DG-DC).POLY(DG-DC) FACILITATES Z-DNA FORMATION.

Moller A, Nordheim A, Nichols SR, Rich A
Dept. Biology, Massachusetts Inst. Technology, Cambridge, MA, 02139
Proc Natl Acad Sci USA; 78(8):4777-4781 1981

Poly(dG-dC).poly(dG-dC) was methylated at the N-7 position of guanine through the use of dimethyl sulfate. The conversion of poly(dG-dC).poly(dG-dC) from B-DNA to the Z-DNA form was followed by measuring both the circular dichroic spectra and changes in the absorbance ratio A295/A260. Increasing methylation steadily decreases the amount of NaCl or MgCl2 that is required to convert the polymer from B-DNA to Z-DNA. At 100% methylation of the guanine residues, the modified polymer is fully converted to Z-DNA in a physiological salt soln. Kinetic experiments show that methylation markedly accelerates the speed of the conversion from B-DNA to Z-DNA in the presence of added salt. These effects may be partly due to the positive charge of guanine that accompanies N-7 methylation. (Author abstract) (20 Refs)

32. IN VIVO KINETICS OF O(6)-METHYLGUANINE AND 7-METHYLGUANINE FORMATION AND PERSISTENCE IN DNA OF RATS TREATED WITH SYMMETRICAL DIMETHYLHYDRAZINE.

Herron DC, Shank RC
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Cancer Res; 41(10):3967-3972 1981

The rates of appearance and removal of 7-methylguanine and O6-methylguanine in DNA from rat liver, kidney, and colon were determined at various intervals up to 120 hr after

ip administration of 10.2, 40.7, 81.5, or 163 mg 1,2-dimethylhydrazine (SDMH) per kg body wt (1/16, 1/4, 1/2, or 1 50% lethal dose) using high-pressure liquid chromatography and fluorescence spectrophotometry. In most cases, increasing doses of SDMH slowed the rate of methylation of DNA, especially of the liver; colon DNA was methylated at a faster rate than was liver DNA, and kidney DNA was methylated at the slowest rate following SDMH administration. Removal of O6-methylguanine was slow (half-life, 37 to 50 hr) when this base was present in liver DNA at concentrations above 400 umol/mol guanine; as the concentration fell below 300 umol O6-methylguanine per mol guanine, the removal rate more than doubled (half-life, 16 to 19 hr). Some evidence was obtained to suggest that in the first 12 hr after max DNA methylation following SDMH administration, a rapid time-dependent removal of 7-methylguanine from liver and kidney but not colon DNA occurred. In these instances, then, the rates of formation and removal of aberrant methylated bases did not follow first-order kinetics. (Author abstract) (33 Refs)

33. ABERRANT METHYLATION OF LIVER DNA IN RATS DURING HEPATOTOXICITY.

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Toxicol Appl Pharmacol; 60(2):334-345 1981

Aberrant methylation of liver DNA as a response to hydrazine was studied. Male Fischer rats were given hydrazine (60 or 90 mg/kg body wt) by intubation. Liver DNA was analyzed using four high-pressure liquid chromatography methods, a paper chromatography method, and UV absorption spectrometry. Hepatotoxic DNA products were identified as 7-methylguanine (7MG) and O6-methylguanine (O6MG). Administration of [methyl-3H]methionine simultaneously with hydrazine produced labeled 7MG and O6MG, indicating that methylation of DNA was mediated by methionine and S-adenosylmethionine. Aberrant methylation of DNA was also produced by administering ethanol (5 g/kg) or carbon tetrachloride (1 g/kg). Evidently such methylation may be a nonspecific response by the liver to chemical injury. (25 Refs)

34. ON THE PECULIARITIES OF METHYLATION OF DNA OF DIFFERENT TISSUES OF RAT'S FETUS AND MATERNAL ORGANISM AFTER 1,2-DIMETHYLHYDRAZINE TREATMENT.

Likhachev AYa, Petrov AS

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Vopr Onkol; 27(7):71-73 1981

A high rate of DNA methylation in the liver and large colon mucosa was registered 6 hr after dimethylhydrazine (SDMH) treatment of rats with 19-day gestations. However, in f etal liver, a low concentration of methylated guanine (7-methyl guanine) derivatives was found, while in large colon and brain, these substances were not detected at all. It is suggested that the resistance of embryonal tissues to the transplacental effect of SDMH is due to the underdeveloped enzymatic system of the fetus. (Author abstract) (8 Refs) (In RUS)

35. ELEVATED TRANSCRIPTIONAL COMPLEXITY AND DECREASE IN ENZYMATIC DNA METHYLATION IN CELLS TREATED WITH L-ETHIONINE.

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Cancer Res; 41(10):4101-4106 1981

This paper analyzes the extent of enzymatic methylation of DNA in P815 cells cultured for several cell cycles in the presence of 1 mM L-ethionine by use of the bacterial restriction enzymes HpaII, MspI, HhaI, HaeII, and AvaI. The results obtained suggest that the extent of methylation of DNA is decreased during L-ethionine treatment and that the degree of this inhibition varies among different methylation sites. The nucleotide sequence complexity of nuclear RNA in P815 cells was analyzed by excess hybridization with tracer amounts of nick-translated nonrepetitive P815 [3H]DNA sequences. About 6.2% of the nonrepetitive sequences form hybrids with nuclear RNA of control cells, whereas 8.8% are complementary to nuclear RNA from L-ethionine-treated cells. Thus, the decrease in enzymatic DNA methylation correlates with the presence of new transcriptional products suggesting that this modification process inversely correlates with the initiation of transcription. (Author abstract) (45 Refs)

36. RESTRICTION ENDONUCLEASE ANALYSIS OF MITOCHONDRIAL DNA FROM VIRUS-TRANSFORMED, TUMOR AND CONTROL CELLS OF HUMAN, HAMSTER AND AVIAN ORIGIN. SEQUENCE CONSERVATION AND INTRASPECIFIC VARIATION.

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Biochim Biophys Acta; 655(2):210-220 1981

This study compares over 70 recognition sites for restriction endonucleases on mtDNAs from various control versus malignant cells, derived from Syrian hamster, chick embryo, viper and human cells, exhibiting a wide spectrum of cellular transformation and tumor histories. Agents for transformation in vitro and in vivo include Rous sarcoma viruses, simian virus 40, polyoma virus and adenovirus. The results show a striking intraspecific sequence homogeneity of different mtDNAs regardless of tissue origin and oncogenic history. mtDNA from human biopsy specimens of tumor versus pathologically normal areas yielded indistinguishable restriction cleavage patterns reflecting either the 'wild-type' form (with 7 restriction endonucleases) or, in one individual, a variant pattern detected with HpaI. The precise position of the HpaI variant site was determined on the physical map of human mtDNA. Additional cleavage sites in the previously reported restriction map of Syrian hamster mtDNA are also presented. It is concluded that (1) mtDNA sequences in higher animal cells are highly conserved in malignant transformation; (2) no evidence for integration of viral sequences in mtDNA is apparent; (3) variant patterns in mtDNA are likely to be intraspecific polymorphisms that pre-exist neoplastic transformation. The possibility is discussed that altered regulatory interaction with the mitochondrial genome, rather than evident changes in mtDNA primary structure, determine anomalous mitochondrial functions in malignant transformation. (Author abstract) (62 Refs)

37. SITE OF LINKAGE BETWEEN ADENOVIRUS TYPE 12 AND CELL DNAS IN HAMSTER TUMOUR LINE CLAC3.

Deuring R, Winterhoff U, Tamanoi F, Stabel S, Doerfler W
Inst. Genetics, Univ. Cologne, Cologne, W. Germany
Nature; 293(5827):81-84 1981

The total DNA of cell line CLAC3, an adenovirus type 12 (Ad12)-induced hamster tumor line carrying 5 copies of the Ad12 genome, was cleaved with restriction endonuclease EcoRI; fragments larger than 4.0 kilobases (fragment D) were selected by velocity sedimentation; fragment D was ligated to the arms of bacteriophage lambda-derivative vector; phage containing DNA sequences derived from the termini of AD12 DNA were selected by using the terminal EcoRI fragments A and C as probes; and recombinant phage were purified and propagated to yield large amounts of recombinant DNA for characterization. Three plaques of recombinant lambda phage containing DNA sequences homologous to the left terminus of Ad12 were isolated, and the DNA from them characterized by numerous techniques, including restriction endonuclease cleavage, Southern blotting, and DNA-DNA hybridization. The nucleotide sequence at the junction between the left terminus of the Ad12 DNA and the hamster cellular DNA is preserved beginning with base pair 46 of the Ad12 DNA, which occupies position 83 of the recloned fragment; the first 82 base pairs of the fragment are not viral but contain scattered 8-to 11-base-pair homologies to distant sequences within the first 2,722 left terminal base pairs of Ad12 DNA. Computer analysis revealed regions of dyad symmetry with only a few mismatched bases between base pairs 35 and 54 and 71 and 90 that straddle the site of the junction and permit the construction of a stemmed loop with 15 matched and 4 unmatched pase pairs in the stem and 16 nucleotides in the loop. The stability of this loop and its possible biological function(s) are still unknown. (23 Refs)

38. DOES SIMIAN VIRUS 40 T ANTIGEN UNWIND DNA?

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Dept. Biochemistry, Univ. California, Berkeley, CA, 94720
J Biol Chem; 256(19):10156-10160 1981

Recently developed techniques allow rapid and accurate determination of protein-induced DNA unwinding. We have used such techniques to determine whether SV40 T antigen unwinds DNA. Assays were performed with purified wild type T antigen, D2 protein, and the SV80 T protein over a wide range of protein concentrations. Additionally, several DNA templates were used, including SV40 DNA, pBR322 DNA, and two plasmid DNAs containing one and three copies of the SV40 origin of replication. None of the purified proteins showed unwinding activity either in the presence or absence of ATP and Mg++. As a positive control, we found that unwinding of pBR322 DNA by Escherichia coli RNA polymerase occurred under identical assay conditions. Protein-DNA interactions that involve unwinding of the helix are sensitive to the superhelical density of the DNA. Using a filter binding assay, we found that the efficiency of the binding of T antigen to the viral origin of replication does not depend upon the topological state of the DNA. This result further supports our conclusion that SV40 T antigen is not an unwinding protein. (Author abstract) (32 Refs)

39. CHROMATIN ANALYSIS IN HUMAN ENDOMETRIAL ADENOCARCINOMA BEFORE AND AFTER TREATMENT WITH 6-METHYL-17-HYDROXYPROGESTERONE ACETATE (MPA).

Barni S, Novelli G, Zanoio L, Gerzeli G, Vecchietti G
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Virchows Arch [Cell Pathol]; 37(2):167-177 1981

Some molecular and ultrastructural characteristics of chromatin (propidium iodide intercalation into DNA during thermal denaturation. DNA content per nucleus and its hydrolysis kinetics, euheterochromatin ratio) were studied in the cells of endometrial adenocarcinomas following oral treatment with 6-methyl-17-hydroxyprogesterone acetate (MPA) at a dose of 1 g/day for 12 days. Ultrastructurally, before treatment the nuclei of tumor cells were often irregularly shaped and exhibited a mostly dispersed chromatin (euchromatin). In addition, the distribution of DNA content per cell was clearly abnormal, reaching ploidy levels of 16c. From a physicochemical standpoint, both the thermal denaturation and the HCl-hydrolysis of DNA revealed a high lability of chromatin. After progestin treatment, nuclear morphology appeared to approach normality, and large areas of heterochromatin reappeared which, after staining with propidium iodide, could be seen as strongly fluorescent points. DNA showed a higher resistance to thermal denaturation, especially evident at temperatures exceeding 70 C. Also the HCl-hydrolysis curves showed that, particularly with regard to short (less than 20 min) or protracted (greater than 140 min) hydrolysis times, DNA was less easily hydrolyzed than before MPA-treatment. A clearcut modification could be observed with regard to the DNA content per cell, high ploidy levels disappearing and values being less dispersed. On the whole, these findings demonstrated an effect of the drug on tumor cells, particularly affecting chromatin, at least with regard to the parameters examined. (Author abstract) (18 Refs)

40. INTERACTION OF THE ANTITUMOR DRUG 4'-(9-ACRIDINYLAMINO)METHANESULFON-M-ANISIDIDE AND RELATED ACRIDINES WITH NUCLEIC ACIDS.

Wilson WR, Baguley BC, Wakelin LP, Waring MJ
Dept. Pathology and Cell Biology, Univ. Auckland Sch. Medicine, Private Bag, Auckland, New Zealand
Mol Pharmacol; 20(2):404-414 1981

The acridine antitumor drug 4'-(9-acridinylamino)methanesulfon-m-anisidide (m-AMSA) is known to be an inhibitor of nucleic acid synthesis. Correlation between m-AMSA (and other related acridine) binding to different nucleic acids (calf thymus) and biological activity and molecular structure were examined using equilibrium dialysis, spectrophotometry, and competition with ethidium. All three techniques indicated an intrinsic association constant of approx 1.5 x 10(5)/M at an ionic strength of 0.01. Increasing ionic strength depressed the association constant by a factor corresponding to that predicted for a monocationic ligand. Binding of m-AMSA was highly selective for native DNA; its affinity for heat-denatured DNA and ribosomal RNA was lower by factors of 5 and 25, respectively. All acridines examined discriminated in favor of DNA Among seven acridines tested, no simple correlation could be found between antitumor potency or selectivity and strength of DNA binding. Presence of an unsubstituted methanesulfonanilide ring did not interfere with binding to double stranded DNA, but addition of a methoxy substituent at the 3' ring position may sterically hinder intercalation of the acridine nucleus of the drug. (36 Refs)

41. DNA SUPERCOILING, SHORTENING, AND INDUCTION OF SINGLE-STRAND REGIONS BY CIS-DIAMMINEDICHLOROPLATINUM(II).

Mong S, Daskal Y, Prestayko AW, Crooke ST
Bristol-Baylor Lab., Dept. Pharmacology, Baylor Coll. Medicine, Houston, TX. 77030
Cancer Res; 41(10):4020-4026 1981

The compound cis-diamminedichloroplatinum(II) (CDDP) was found to bind to covalently closed circular supercoiled, covalently closed circular nonsupercoiled, and single-strand broken relaxed PM2 DNA and induce different types of DNA conformational changes. Using Kleinschmidt's technique, it was found that binding of CDDP decreased the DNA length to 75% of the original single-strand broken relaxed DNA without inducing superhelical conformational changes. CDDP also shortened the length of covalently closed circular nonsupercoiled DNA before a supercoiled conformation was generated. Single strand-specific nucleases were used to detect drug-induced DNA structural alterations. Local single-strand regions or regions of denaturation were detected by S1 nuclease from Aspergillus oryzae and by BAL-31 nuclease from Alteromonas espejiana BAL-31. The single-strand regions or local denaturation regions do not seem to be related to or caused by DNA superhelical conformational changes since they were detected at drug concentrations at which no significant DNA superhelical turns were found. DNA shortening, superhelical conformational changes, and local denaturation regions can be explained by the previously proposed 'DNA intrastrand cross-linking' model. (Author abstract) (31 Refs)

42. DNA-PROTEIN CROSS-LINKING BY CHROMIUM SALTS.

Fornace AJ, Seres DS, Lechner JF, Harris CC
Lab. Experimental Pathology, Div. Cancer Cause and Prevention, NCI, NIH, Bethesda, MD, 20205
Chem Biol Interact; 36(3):345-354 1981

DNA-protein cross-links were detected in several types of mammalian cells in culture when they were exposed to chromate salts. The cell types included human bronchial epithelial cells - the apparent cell type of origin of the malignancies reported in chromate workers. The level of cross-linking was proportional to the concentration of chromate used. These cross-links appeared to be persistent since no removal was seen after 12 hr of repair incubation. A low level of DNA single strand breaks (SSB) were also induced after exposure of the cells to chromate but were rejoined after 4 hr of repair incubation. The active form of chromium appears to be the trivalent since chromic but not chromate salts induced DNA-protein cross-links in isolated nuclei. Chromic salts also produced cross-linking between DNA and protein in soln while the hexavalent form was inactive. These data imply that chromate crosses the cell membrane, is reduced to the trivalent form and induces stable linkages of DNA to protein. (Author abstract) (19 Refs)

OTHER RELATED STUDIES

43. EFFECT OF PRETREATMENT WITH PREGNENOLONE-16ALPHA-CARBONITRILE AND PROTEIN-FREE DIET ON THE METABOLISM AND CARCINOGENICITY OF DIMETHYLNITROSAMINE.

Kiessling M, Lipinski R, Bohm N, Kleihues P
Pathologische Institut, Universitat Freiburg, 78 Freiburg, W. Germany
Carcinogenesis; 2(8):757-761 1981

Adult female Wistar rats were pretreated with a protein-free diet (PFD), pregnenolone-16alpha-carbonitrile (PCN: total dose 25 mg, 5 equal doses at 12-hr intervals), or a combination of both. Rats from each group were given a single injection of 60 mg dimethylnitrosamine (DMN). PCN-pretreated rats showed a 50% tumor incidence after a mean survival time of 672 days. PFD (30 rats) and PFD + PCN (28 rats) groups showed a tumor incidence of 83-85% and a mean survival time of 357 and 396 days. Rats treated only with PCN showed a tumor incidence of 50% with a mean survival time of 805 days. The incidence of neoplasms, particularly cholangiomas, was highest following treatment with PCN only. The PFD and PFD + P CN groups showed lower incidence of cystic cholangiomas and no malignant liver tumors. Methylation of liver DNA purines by a single dose of [14C] DMN was greatest in the PCN and PFD groups and lower in the PFD + PCN group. Methylation of renal DNA was highest in the PFD and PFD + PCN groups and lower in the PCN-only pretreated group. Differences in lung tissue were smaller among the various groups. Both PCN and PFD reduced the hepatotoxicity of DMN. PCN had no effect on the induction of renal tumors. Apparently PCN does not cause a reduction in DNM metabolism in the liver and the resultant increase in DNA methylation in the kidney. PFD shifts the metabolism of DMN from the liver to the kidney, lowering the carcinogenic effect of DMN in the liver and raising it in the kidney. (24 Refs)

44. ISOLATION AND CHARACTERIZATION OF POLYPLOID NUCLEI OF R3230AC MAMMARY ADENOCARCINOMA AND COMPARISON WITH NORMAL MAMMARY NUCLEI.

Kulick D, Liu DK
(c/o Liu) Dept. Pharmacology and Specialized Cancer Res. Center, Milton S. Hershey Medical Center, Pennsylvania State Univ. Coll. Medicine, Hershey, PA, 17033
Cancer Res; 41(10):3907-3912 1981

The R3230AC mammary adenocarcinoma is biochemically similar to the normal lactating mammary gland in many ways, including the RNA/DNA ratio. However, the tumor differs from normal in important ways, which include having a DNA content 2 to 4x higher than the max value for normal mammary gland. This raises the question of whether the cells of the tumor all have more DNA than normal cells. In order to determine how tumor DNA is distributed and whether its transcriptional activity differs from normal, we have compared the ploidy and RNA polymerase activities of nuclei isolated from both sources. Nuclei were also examined for their content of Ca++-stimulated RNase activity, a marker of mammary differentiation. Sucrose density gradient centrifugation of tumor cell nuclei revealed three nuclear types, designated T1, T2, and T3, representing 25, 48, and 27% of total nuclei isolated. T1, T2, and T3 nuclei contained, respectively, 6.5, 12.3, and 19.0 pg DNA per nucleus, representing diploid, tetraploid, and hexaploid amounts of DNA. Nuclear RNA and protein increased in proportion to ploidy. By contrast, cell nuclei isolated from

lactating mammary gland formed only two bands (M1 and M2), comprising 54 and 46%, respectively, of total nuclei isolated. Both M1 and M2 nuclei contained diploid amounts of DNA. Therefore, the higher DNA content in the tumor is attributable, at least in part, to the presence of polyploid nuclei in the majority of tumor cells. The combined activity of DNA-dependent RNA polymerases I, II, and III per nucleus was the same in T1, T2, and T3; M1 and M2 also had activities similar to each other but much lower than the tumor types. Activity attributable to poly merase I (resistant to 200 ug alpha-amanitin per ml), polymerase II (inhibited by 0.06 ug alpha-amanitin per ml), and polymerase III (inhibited by 200 ug alpha-amanitin per ml) was about 35, 50, and 15%, respectively, for each class of tumor nuclei. The corresponding values for M bands were about 44, 34, and 22%. These results suggest that tumor cells synthesize more heterogeneous RNA than do mammary cells of lactating rats, but the latter synthesize more ribosomal RNA. Activity of mammary-specific Ca++-stimulated RNase increased slightly per nucleus as the ploidy of tumor nuclei increased, but in every T group the activity was less than one-half of that of M1 or M2 nuclei. Thus, despite differences in ploidy, all tumor nuclear classes show a lesser degree of differentiation than nuclei from the lactating gland, with respect to their capacity for synthesis of ribosomal RNA compared to messenger RNA and their expression of Ca++-stimulated RNase. (Author abstract) (31 Refs)

45. THE RELATIONSHIP BETWEEN LYMPHOCYTE NUCLEAR MORPHOLOGY AND CELL CYCLE STAGE IN LYMPHOID NEOPLASIA.

Diamond LW, Weisenburger DD, Rappaport H
Dept. Pathology, Temple Univ. Sch. Medicine, Philadelphia, PA, 19140
Am J Hematol; 11(2):165-173 1981

A significant percentage of lymphoid cells isolated from three lymphoid neoplasms (nodular lymphoma mixed cell type, mycosis fungoides, and Sezary syndrome) had tetraploid or near-tetraploid DNA content, as determined by flow cytometric analysis. In each case, a morphologically distinct population of cells with large, irregular nuclei was present, the percentage of which corresponded to the percentage of tetraploid cells. In a fourth case (diffuse 'histiocytic' lymphoma), cells were sorted on the basis of DNA content from the G0-G1 compartment and compared morphologically to cells sorted from the S-G2 (M) compartment. The G0-G1 cells had clumped nuclear chromatin, lacked prominent nucleoli, and had a mean nuclear diameter of 10.18 +-1.22 um. The sorted cells with S-G2(M) DNA content had one to several prominent nucleoli and less clumped nuclear chromatin, and they were significantly larger, with a mean nuclear diameter of 13.76 +-1.59 um (p less than 0.00001). These preliminary results suggest that, in lymphoid malignancies, certain morphologic characteristics of individual lymphoid cells are related to their DNA content (level of ploidy) and/or the stage of the cell within its division cycle. (Author abstract) (18 Refs)

46. 5-FLUOROURACIL INCORPORATION INTO HUMAN BREAST CARCINOMA RNA CORRELATES WITH CYTOTOXICITY.

Kufe DW, Major PP
Sidney Farber Cancer Inst., 44 Binney St., Boston, MA, 02115
J Biol Chem; 256(19):9802-9805 1981

We demonstrate a highly significant relationship (p less than 0.0001) between the incorporation of 5-fluorouracil in total cellular RNA and loss of clonogenic survival of the huma n MCF-7 breast carcinoma cell line. The extent of 5-fluorouracil incorporation in RNA is concentration- and time-dependent. Identical results are obtained in experiments employing thymidine to bypass the block of thymidylate synthetase and reverse inhibition of DNA synthesis. These studies suggest that the incorporation of 5-fluorouracil in RNA is the major mechanism of cytotoxic action in this human cell line. (Author abstract) (24 Refs)

47. MANIFESTATION OF CARCINOGENESIS AS A STOCHASTIC PROCESS ON THE BASIS OF AN ALTERED MITOCHONDRIAL GENOME.

Neubert D, Hopfenmuller W, Fuchs G
Institut fur Toxicologie und Embryopharmakologie, Freien Universitat Berlin, Garystrasse 9, D-1000 Berlin 33, W. Germany
Arch Toxicol; 48(2/3):89-125 1981

Computer calculations are used to show the feasibility of a concept which explains the manifestation of a pathological cell function from a latent state by the phenomenon of extrachromosomal inheritance (through the mitochondrial genome) in mammalian cells. A hypothesis is submitted in which this principle is applied to the process of carcinogenesis. According to this concept, the manifestation of a tumor cell - after the initiation stage - entirely depends on stochastic events, ie, random distribution of mitochondria during cell divisions, with an accumulation of the lesion in a few out of many cells. We feel that this concept comprises a better explanation of many characteristics and peculiarities of the phenomenon of carcinogenesis than do attempts which explain tumor formation as a phenomenon caused by mutation in a nuclear genome. A consideration of the principles presented automatically leads to a number of specific consequences with regard to carcinogenesis. Some of these consequences are discussed. They include: 1. the process of malignant transformation should not be irreversible for all the cells of a progeny; 2. the number of mitochondria in a cell type should be inversely correlated to tumor frequency; 3. the latent period should mainly be determined by the cell division rate and the 'extent' of the initiating event; 4. susceptibility to carcinogenesis may be substantially higher if the number of mitochondria per cell line is increasing or decreasing, ie, during the embryonic and fetal periods; 5. heterogeneous types of cells may arise from a single 'initiated' cell, and 6. the process of malignant transformation should not necessarily be confined to one generation of the species. In addition, experimental approaches to support the submitted concept are suggested. (Author abstract) (91 Refs)

48. FRAMESHIFT MUTAGENESIS OF 9-AMINOACRIDINE DERIVATIVES IN SALMONELLA TYPHIMURIUM.

Young PR, Ma RI, Marfey P, Kallenbach NR
Inst. Cancer Res., Fox Chase Cancer Center, Philadelphia, PA, 19111
Mutat Res; 90(1):1-10 1981

A number of 9-aminoacridine derivatives with various substituents on the exocycl ic amino group were tested in the absence of activation by rat-liver microsomes in order to determine the effect of the substituents on frameshift activity and specificity. The four acridine derivatives tested were 9-aminoacridine, a spin-labeled 9-aminoacridine derivative (4-(9-acridinylamino)-2,2,6,6-tetramethyl-1 piperidinyloxy), quinacrine dihydrochloride, and the putative bisintercalator N,N'-bis(2-methoxy-6-chloroacridine)-tetraethyl enephetamine pentahydrochloride. Frameshift mutagenesis of the acridines was sequence-specific in that significant activity occurred only with the two

strains with a +1 frameshift his C (Salmonella typhimurium strains TA1537 and TA1977) and not with the two strains in which reversion was required at a +2 frameshift in his D (TA1538 and TA1978). Excision repair via uvr B was involved only to a minor extent in the production of a frameshift reversion at the his C locus by the four compounds tested. The dose-response of frameshift reversion at his C induced by the acridine was nonlinear. These results are consistent with a model derived from nuclear magnetic resonance experiments on 9-aminoacridine binding to dinucleoside phosphates, in which the N-H group is situated in the duplex so as to participate in a hydrogen bond with one base while excluding its complementary partner, thereby provoking mismatching. (18 Refs)

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MECHANISMS OF DNA REPLICATION

1. METAL-INDUCED INFIDELITY OF DNA SYNTHESIS.

Zakour RA, Kunkel TA, Loeb LA
Joseph Gottstein Memorial Cancer Res. Lab., Dept. Pathol., Univ. Washington, Seattle, WA, 98195
Environ Health Perspect; 40:197-205 1981

Present state of knowledge, public health implications, and required research are reviewed. Topics discussed include mechanism of DNA polymerization, fidelity of DNA synthesis, assays with polynucleotide templates, metal activators and non-activating metal ions and fidelity, screening for metals that alter fidelity, fidelity of DNA synthesis with natural DNA templates, and mechanism of genetic miscoding by metals. A significant correlation exists between metals that decrease fidel-

HE 20.3173/2:

ity and those r eported to be mutagenic and carcinogenic. To date greater than 40 metal compounds have been tested in graded concentrations for effects on fidelity of DNA synthesis. Compounds which increase infidelity by greater than 30% at two or more concentrations are scored positive: these included all the known mutagens/carcinogens (Ag, Be, Cd, Co, Cr, Mn, Ni, Pb), although evidence in the literature is equivocal for three metals. Of these, Cu++ increased misincorporation and Fe++ and Zn++ did not alter fidelity. With few exceptions these results have been confirmed. The general postulate that carcinogens can be identified by their interactions with DNA includes metals. (37 Refs)

2. MECHANISMS OF TOLERANCE TO DNA LESIONS IN MAMMALIAN CELLS.

Meneghini R, Menck CF, Schumacher RI
Dept. Biochemistry, Inst. Chemistry, Univ. Sao Paulo, C.P. 20780, Sao Paulo, Brazil
Q Rev Biophys; 14(3):381-432 1981

The way in which mammalian cells deal with a large burden of unexcised DNA lesions is discussed. The biological consequences of unexcised DNA lesions are considered in terms of cell killing, cell mutation, cell transformation, enhanced virus reactivation, and sister chromatid exchanges. These different biological effects seem to be triggered during DNA replication. Thus, the damaged template has to be replicated in order for the chain of events leading to a certain effect to be initiated. DNA replication on a damaged template is discussed in relation to information from bacterial systems, DNA containing lesions replicated in mammalian cells, effect of template-strand lesions on the rate of DNA synthesis and the structure of the daughter strand, inhibition of replication fork by lesions in the template strand, DNA synthesis along the lesions (postreplication repair), and mechanisms of postreplication repair (recombination, branch migration, 'translesion synthesis', and removal of blocked sites by joining of adjacent replicons). Hints of the inducibility of tolerance to DNA lesions come from distinct approaches such as the enhancement of DNA chain elongation, recovery of the rate of DNA synthesis, recovery in survival, enhanced virus reactivation, and induction of proteins by DNA-damaging agents. (163 Refs)

EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

3. THE EFFECT OF ADDED H1 HISTONE AND POLYLYSINE ON DNA SYNTHESIS AND CELL DIVISION OF CULTURED MAMMALIAN CELLS.

Kundahl E, Richman R, Flickinger RA
Dept. Biological Sciences, State Univ. New York at Buffalo, Buffalo, NY, 14260
J Cell Physiol; 108(3):291-298 1981

The effect of adding whole histone, various histone fractions, polylysine, and polyarginine on the rate of cell division and DNA synthesis of three murine cell lines (cultured Friend erythroleukemia cells and two mouse lymphoma cell lines, el-4 and S-49) was studied. Low levels (10 ug/ml) of H1 histone or polylysine stimulated DNA synthesis by the creation of additional initiation sites for replication and production of smaller av replicon sizes. Untreated Friend erythroleukemia cells underwent a decrease in cell division rate on days 2-5 of culture while the decrease in rate of cell division was much less for cells treated with H1 histone or polylysine. This might mean that the observed results are not due to the stimulation of cell division but rather to a continuation of cell division in the H1 and polylysine-treated cultures. However, the data from the experiments with the lymphoma cells showed clearly that H1 histones can stimulate the rate of cell division before the rate of cell division of the untreated cells began to plateau. In the lymphomas, there was a stimulation of the rate of cell division after 1 day of cultre and a slight stimulation of cell division of the Friend cells after 2 days of culture. These data indicated that there is not a long lag before H1 histone and polylysine affect cell division and that these compounds are not acting merely by affecting the level of depletion of some material in the culture medium after a prolonged period of culture. DNA fiber autoradiographic experiments revealed that replicon size is decreased in the cells cultured with H1 histone and polylysine at later periods of culture. (25 Refs)

4. MECHANISM OF SYNERGISTIC INDUCTION OF DNA SYNTHESIS BY EPIDERMAL GROWTH FACTOR AND TUMOR PROMOTERS.

Matrisian LM, Bowden GT, Magun BE
Dept. Anatomy, Univ. Arizona Health Sciences Center, Tuscon, AZ, 85724
J Cell Physiol; 108(3):417-425 1981

The effects of a number of phorbol ester compounds with varying tumor-promoting abilities on epidermal growth factor (EGF) binding, degradation, and stimulation of DNA synthesis was studied in the Rat-1 cell line (F2408 established line derived from Fischer rat embryo fibroblasts). The phorbol esters alone were able to induce a small increase in the amount of DNA synthesis compared to Me2SO-treated control cells. In combination with high concentrations of EGF (1-10 nanog/ml), the effect of phorbol esters on EGF-induced DNA synthesis was less than additive. However, at EGF concentrations between 0.001 and 0.05 nanog/ml, there was a marked synergism between EGF and phorbol esters. 12-O-tetradecanoyl phorbol-13-acetate (TPA) exhibited the greatest degree of synergism at low EGF concentrations. The observed increase in DNA synthesis in the presence of TPA and EGF was due to a TPA-induced increase in the overall rate of synthesis. The inhibition of EGF binding to Rat-1 cells by TPA and other phorbol ester tumor promoters was dose dependent. The ability of the various phorbol esters to spare EGF from degradation and to act synergistically with EGF to induce DNA synthesis correlated with the tumor-promoting ability of the compounds. In addition, the concentration of EGF at which there was a significant increase in the amount of EGF spared from degradation in phorbol ester-treated cells, compared to controls, correlated with the EGF concentration at which EGF and phorbol esters exerted synergistic effects on DNA synthesis. (31 Refs)

5. BLEOMYCIN INDUCED INHIBITION OF DNA SYNTHESIS IN ATAXIA-TELANGIECTASIA CELL LINES.

Edwards MJ, Taylor AM, Flude EJ
Cancer Res. Campaign Lab., Dept. Cancer Studies, Medical Sch., Univ. Birmingham, Birmingham B15 2TJ, England
Biochem Biophys Res Commun; 102(2):610-616 1981

Inhibition of DNA synthesis by bleomycin (30-300 ug/ml) was studied in fibroblast and lymphoblastoid cells from normal individuals and lymphoblastoid cells from ataxia-telangiectasia (AT) patients. DNA synthesis was measured by incorporation of [14C]thymidine. In normal cells bleomycin produced a biphasic inhibition of DNA synthesis which increased sharply

with bleomycin dose at low dose levels and leveled off at higher doses. The first, sharp increase in inhibition may be due to inhibition of replicon initiation, and the second, more shallow component may result from inhibition of chain elongation. Inhibition of DNA synthesis was much slower in cells from AT patients than in normal cells at bleomycin doses less than 100 ug/ml. Low and high mol wt DNA fractions were not altered in AT cells. Replicon initiation and chain elongation were inhibited only at bleomycin doses of 100 ug/ml or more. Results suggest a defect in repair of bleomycin-caused damage in AT cells. (24 Refs)

6. THE INHIBITION OF DNA REPLICATION IN ATAXIA TELANGIECTASIA CELLS AFTER INTRODUCTION OF DNA DAMAGE (MEETING ABSTRACT).

Jaspers NG, De Wit J, Bootsma D
Laboratory of Cell Biological and Genetics, Erasmus University Rotterdam, Rotterdam, The Netherlands
Symposium on Host Factors in Human Carcinogenesis, June 8-11, 1981, Cape Sunion, Greece International Agency for Research on Cancer; 1981

When cultured fibroblasts or phytohemagglutinin-stimulated lymphocytes from patients with ataxia telangiectasia (A-T) are exposed to ionizing radiation, an inhibition of DNA synthesis is observed. This inhibition is, however, less pronounced than in identically treated normal cells. In all A-T cell strains tested this abnormal behavior was found, independent of their capacity to repair gamma-ray induced DNA damage. The dimished DNA synthesis inhibition was also evident after exposure to bleomycin or to x-rays delivered under anoxic conditions. A normal response was seen after treatment with UV light of 254 nm, alkylating agents or mitomycin C. In contrast, two A-T cell strains that have been shown hypersensitive to the killing action of 4-nitroquinoline-1-oxide, exhibited a DNA replication inhibition significantly stronger than normal cells or A-T cells with normal sensitivity to the drug. Cells from A -T heterozygotes showed near-normal rates of DNA replication after exposure to x-rays or bleomycin. Utilizing the difference in DNA replication rate between X-irradiated A-T and normal cells, a procedure was developed for genetic complementation analysis of A-T strains. In addition to the complementation groups A and B described earlier by Paterson and coworkers, we have identified two new groups (C and D). These data indicate an extensive genetic heterogeneity in A-T, comparable to that found in xeroderma pigmentosum. (no Refs)

7. THE 2-5A (PPPA2'P5'A2'P5'A) SYSTEM IN INTERFERON-TREATED AND CONTROL CELLS (MEETING ABSTRACT).

Kerr IM, Wreschner DH, Cayley PJ, Silverman RH, Knight M, Gilbert CS, Brown RE
Imperial Cancer Research Fund Laboratories, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, England
Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

The unusual oligonucleotide pppA2'p5'A2'p5'A (and related oligmores collectively referred to as 2-5A) was discovered on the basis of its role in the interferon-mediated inhibition of cell-free protein synthesis by double-stranded RNA (dsRNA). Both in the intact cell and cell-free system 2-5A is unstable and the activation of the nuclease is transient. Nevertheless, 2-5A introduced into intact cells inhibits protein and DNA synthesis and virus growth. It also occurs naturally in interferon-treated encephalomyocarditis virus-infected cells in amounts consistent with its playing a part in the interferon-mediated inhibition of virus growth. The 2-5A synthetase is widely distributed in a variety of cells and tissues at levels which vary with both growth and hormone status. In addition the non-phosphorylated 'core' of 2-5A system may, however, have a wider significance than simply in the antiviral action of interferon. The 2-5A (A2p) nA has been reported to ***inhibit DNA synthesis*** and cell growth. The natural occurrence of core has not been described previously. The 2-5A dependent nuclease has been partly purified from rabbit reticulocytes and Ehrlich ascites tumor cells. It shows a considerable degree of sequence specificity in its cleavage of RNA. Accordingly the specificity of the enhanced endonucleolytic cleavages observed in the intact interferon-treated cell are currently under investigation. The role of the 2-5A system in the antiviral and antigrowth activities of interferon and its possible wider significance will be discussed in the light of these results. (3 Refs)

8. IN VITRO INHIBITORY EFFECT OF INTERFERON ON DNA SYNTHESIS OF BREAST CANCER CELLS AND RELATIONSHIP TO STEROID RECEPTORS (MEETING ABSTRACT).

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Eur Surg Res; 13(1):31 1981

For selecting patients for postoperative interferon treatment we carried out the following in vitro study. From 22 patients with primary or recurrent breast cancer, fresh tumor tissue was obtained. Tissue cultures were prepared from mouse bone marrow cells according to the method of Dexter et al, and used as a feeder layer for cultivation of breast cancer cells in a humified atmosphere. At least 48 hr after incubation, confluent tumor cell colonies could be determined and the cells could be differentiated as epithelial using electron microscopy. For determining the influence of interferon DNA synthesis 3H-thymidine uptake was measured after incubation of breast cancer cells with interferon or without for control. Human lymphoblast interferon (HLI) was obtained from Namalwa cells after induction by Sendai virus. Human fibroblast interferon (HFI) was induced in human fibroblasts by Poly I:C and purified by controlled pore glass chromatography. Ten and 100 U/ml interferon were used. Forty-eight hr after incubation with interferon 3H-thymidine was measured. (1) An optimal reduction of DNA synthesis could be achieved in 32% with 100 U/HFI and in 62% with the same dose of HLI. (2) Using only 10 U interferon, only suboptimal reductions were seen. (3) 22% of ER+ culture cells show a marked reduction of DNA synthesis compared with 50% ER cells. These results indicate that 100 U HLI is more effective than the same dose of HFI. The higher response rate of cells or ER tumors could be an argument for selecting ER patients for interferon therapy. (3 Refs)

9. EFFECTS OF DEOXYNUCLEOSIDES ON CULTURED HUMAN LEUKEMIA CELL GROWTH AND DEOXYNUCLEOTIDE POOLS.

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Cancer Res; 41(11,part1):4493-4498 1981

We investigated the mechanism of cell growth inhibition caused by the deoxyribonucleosides thymidine (dThd), deoxyguanosine (dGuo), deoxyadenosine (dAdo), and deoxycytidine (dCyd). Growth of the cultured human leukemic cells HL-60 and K-562 was measured by cloning in soft agar. Of the deoxyribonucleosides, dGuo was the most potent cell growth

inhibitor; however, the potency of added dAdo was probably attenuated by the presence of adenosine deaminase in the tissue culture growth medium. The concentrations of nucleoside causing 50% inhibition of HL-60 cloning were: dCyd, greater than 10,000 uM; dAdo, 500 uM; dThd, 5,000 uM; and dGuo, 80 uM. For K-562 cloning, the concentrations causing 50% inhibition of cloning were dCyd, greater than 10,000 uM; dAdo, 1,600 uM; dThd, 880 uM; and dGuo, 100 uM. Measurement of deoxycytidine 5'-triphosphate (dCTP) pool size in HL-60 cells following incubation with 750 uM deoxyribonucleosides reveal ed that dGuo caused the greatest reduction of dCTP pools, both in early (passage 10)- and late (passage 71)-passage-derived HL-60 cell cultures (35 and 19% of control, respectively), compared to dThd (61 and 26% of control, respectively) and dAdo (39% of control of HL-60 passage 10). In K-562 cells, reductions in dCTP pool size caused by dAdo, dThd, and dGuo were 67, 46, and 35% of control, respectively. Incorporation of [3H]dCyd into DNA of HL-60 and K-562 cells was enhanced by dThd and dGuo, but the degree of enhancement was greater for dThd than for dGuo. Despite its effect in reducing HL-60 dCTP pool size, dAdo failed to enhance [3H]dCyd incorporation in either HL-60 or K-562 cells. Addition of dCyd to the cultures could only partially rescue the inhibition of HL-60 cloning caused by dThd or dGuo, suggesting that inhibition of cytidine 5'-diphosphate reduction by ribonucleotide reductase is not the only mechanism whereby these nucleosides inhibit leukemic cell cloning. These data suggest that, in addition to inhibiting de novo dCTP production via ribonucleotide reductase, these nucleosides may affect other processes in the salvage pathway such as cellular uptake and phosphorylation or the DNA polymerase reaction itself. (Author abstract) (40 Refs)

10. THE UTILIZATION OF TRIFLUOROTHYMIDINE (TFT) TO SELECT FOR THYMIDINE KINASE-DEFICIENT (TK-/-) MUTANT FROM L5178Y/TK+/- MOUSE LYMPHOMA CELLS.

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Mutat Res; 85(5):363-378 1981

Trifluorothymidine (TFT) was analyzed for its ability to select for thymidine kinase-deficient mutants among the cultured L5178Y mouse lymphoma cell lines TK(+/-)-3.7.2C and TK(-/-)-3.7.2C.100. A TFT dose of 1 ug/ml was a more effective and versatile agent for TK(-/-) mutants arising from the TK(+/-) heterozygote than 50 ug/ml bromodeoxyuridine (BUdR), the traditional selective agent. The BUdR-phosphorylated derivative must be extensively incorporated into DNA in order to select against TK competent cells, whereas the TFT derivative acts by quickly inhibiting thymidylate synthetase and arresting DNA synthesis by depleting the thymidylate pool. TFT arrested TK-competent cell growth before a single cell division; BUdR allows several cell divisions, resulting in a dense background haze of microcolonies. (28 Refs)

11. MODE OF ACTION STUDIES OF THE ANTITUMOR ANTIBIOTIC TOROMYCIN AND THE RELATED COMPOUND CHRYSOMYCIN (MEETING ABSTRACT).

Wei TT, Byrne K, Elespuru R, Greenstein M, Pickle D, Richardson C, Stroshane R, Weiss U, White RJ

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Program and Abstracts of the 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, November 4-6, 1981, Chicago, Illinois American Society for Microbiology; :A55 1981

The mechanism of action of toromycin, a new antitumor antibiotic, and the closely related compound chrysomycin was investigated. Macromolecular synthesis studies in Bacillus subtilis indicated that upon addition of toromycin or chrysomycin, nucleic acid synthesis was inhibited preferentially to protein synthesis. In particular, inhibition of DNA synthesis was more complete than RNA synthesis. Both toromycin and chrysomycin demonstrated strong activity in the biochemical prophage induction assay (BIA), a sensitive test for agents interacting with DNA. Interaction of these compounds with DNA in vitro was detected by an acridine orange competitive fluorescence polarization assay. Agarose gel electrophoresis of supercoiled DNA indicated that toromycin binds by intercalation at concentrations less than 5 ug/ml. Our data suggest that drug interaction with DNA plays a prominent role in the biological activity of this new type of antitumor antibiotic. (no Refs)

12. REVERSIBLE INHIBITION OF RAT HEPATOCYTE PROLIFERATION BY HYDROCORTISONE AND ITS EFFECT ON CELL CYCLE-DEPENDENT HEPATOCARCINOGENESIS BY N-METHYL-N-NITROSOUREA.

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Cancer Res; 41(11,part1):4653-4660 1981

The susceptibility of hepatocytes to carcinogenesis in vivo may be influenced by the phase of the cell cycle at which carcinogen-induced damage is incurred. In order to better understand this relationship, hepatic cell proliferation in juvenile male Fischer 344 rats was charted following a two-thirds partial hepatectomy. For hepatocytes, two distinct waves of ***DNA synthesis*** occurred which were followed after 6 to 8 hr by waves of mitotic cell division. In contrast to this kinetic pattern, when hydrocortisone was given after the partial hepatectomy, the initial waves of DNA synthesis and mitosis by hepatocytes were each delayed by about 15 hr. In rats not given hydrocortisone, susceptibility to hepatocarcinogenesis by N-methyl-N-nitrosourea was greatest at 20 hr after partial hepatectomy when the peak fraction of proliferating hepatocytes was in the S phase. By shifting the time of onset of DNA synthesis, the hydrocortisone treatments also shifted the time with greatest sensitivity to N-methyl-N-nitrosourea, with hepatocytes in late G1 or S again the most susceptible. Numerous tumors were also induced by N-methyl-N-nitrosourea in extrahepatic tissues, including intestine, Zymbal's gland, nervous system, kidney, odontogenic tissues, and peritesticular mesothelium. The results illustrate the importance of cell proliferation in carcinogenesis and further point to the specific sensitivity of certain cell cycle phases. (Author abstract) (31 Refs)

13. PROTECTION OF HEMOPOIETIC TISSUE IN WHOLE BODY GAMMA IRRADIATED MICE BY CYSTAMINE GIVEN INTRAMUSCULARLY.

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Radiobiol Radiother (Berl); 22(3):315-317 1981

Protection of hemopoietic cells by cystamine was tested in male white SPF mice aged 10-12 wk. Cystamine (150 mg/kg body wt) was injected ip or im. Controls received ip or im injections of distilled water in the same vol as the cystamine injections. Ten min after injection the mice were exposed to 0.462 Gray/min gamma radiation from a 60Co source for a total dose of 8 Gray. Endogenous spleen colonies and the incorporation of radioiron were evaluated. DNA synthesis in the spleen was measured by the incorporation of 125I-labeled iodouridine. Both ip and im injections of cystamine protected hemopoietic stem cells in a similar manner. Protected animals showed an increase in 59Fe and 125I uptake, but the difference ip-injected mice and controls was not significant. Im injection was more effective than ip injection. Uptake of 59Fe and 125I was significantly higher in im-injected animals than in controls. The higher effect of im over ip injection was confirmed. (7 Refs)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

14. HISTOCHEMICAL DEMONSTRATION OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE IN LEUKEMIA.

Hecht T, Forman SJ, Winkler US, Santos S, Winkler KJ, Carlson F, Maslow WC, Borer WZ, Blume KG
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Blood; 58(4):856-858 1981

WBC from 22 patients with leukemia and lymphoma were studied for the presence of terminal deoxynucleotidyl transferase with a peroxidase-antiperoxidase method. The enzyme was detected in leukemic cells of 5 patients with acute lymphoblastic leukemia and 1 patient with chronic myelogenous leukemia, whereas 16 patients with different forms of leukemia or lymphoma were negative for this enzyme. Comparative studies using a biochemical and an indirect immunofluorescence assay revealed complete concordance between these three methods. (Author abstract) (11 Refs)

15. CLINICAL UTILITY OF LEUKEMIA CELL TERMINAL TRANSFERASE MEASUREMENTS.

McCaffrey R, Lillquist A, Sallan S, Cohen E, Osband M
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Cancer Res; 41(11,part2):4814-4820 1981

Interest in the DNA-synthetic enzyme terminal deoxynucleotidyl transferase (TdT) has developed from two sets of observations: first, in normal animals, it occurs only in immature thymic lymphocytes and in a subpopulation of bone marrow lymphocytes: second, it is present in the blast cells of almost of all patients with acute lymphoblastic leukemia. A prospective trial to evaluate blast cell Td T as a predictor of responsiveness to vincristine and prednisone in 30 Philadelphia chromosome-positive patients with blastic chronic myelogenous leukemia was undertaken. Eleven of 16 TdT-positive patients responded, whereas only 1 of 14 TdT-negative patients showed improvement. Among TdT-positive patients under the age of 50 yr, the response rate was 78%. Enzyme-negative patients under the age of 50 had an 11% response rate. Blast cell morphology (ie, lymphoblastic vs myeloblastic) had no significant correlation with either responsiveness or TdT activity. These results suggest that blast cell TdT activity may identify leukemic patients who are likely to respond to vincristine and prednisone irrespective of their conventional classification. (Author abstract) (49 Refs)

16. METHODS AND CLINICAL RELEVANCE OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE DETERMINATION IN LEUKEMIC CELLS.

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Haematol Blood Transfus; 26:68-72 1981

Current technology for the demonstration of terminal deoxynucleotidyl transferase (TdT) is reviewed. The significance of TdT in the clinical and pathophysiologic evaluation of human leukemias and lymphomas is discussed. In acute leukemias, the highest levels of TdT activity are found in approx 95% of non-T, non-B, and T cell acute lymphoblastic leukemias. Elevated levels of TdT have also been observed in some patients with acute nonlymphoblastic leukemia. All myeloproliferative syndromes (MPS: polycythemia vera and chronic myeloid leukemia) and myelodysplastic syndromes (MDS) were found to be TdT- during the chronic phase of their disease; approx 30% of blast phase chronic myeloid leukemias and 15% of other MDS and MPS terminating in acute leukemia were found to exhibit high levels of TdT activity. In patients with lymphoblastic lymphomas of the T and null cell type, high levels of TdT were observed. Lymphoproliferative diseases of the mature T and B cell type have been found to be TdT-. The significance of TdT markers for diagnosis, therapy planning, and prognosis is discussed. The relationship of TdT determinations to monitoring of disease activity in TdT+ neoplasia is considered. The determination of TdT activity and definition of cell phenotypes in hematopoietic malignancies may be useful tools for classification of these disorders and may have significant prognostic, clinical, and pathogenetic implications. (17 Refs)

17. INHIBITORY EFFECTS OF ANTI-NEOPLASTIC PLATINUM COMPLEXES ON DNA AND RNA POLYMERASES (MEETING ABSTRACT).

Kohl HH, Miller DL
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Clin Chem; 27(6):1066 1981

The present work studies the inhibitory actions of cis-diaminedichloroplatinum II (cis-DDP), trans-diaminedichloroplatinum II (trans-DDP) and other more recently synthesized platinum complexes on both DNA and DNA-dependent RNA polymerases. Soluble and purified enzymes from Escherichia coli were studied in the presence of either a calf-thymus DNA template or Poly (dA-dT). We have also studied a completely mammalian transcription system (intact rat liver nuclei) in a DNA-dependent RNA polymerase assay. Measurements of RNA transcription were made using radioactive guanosine triphosphate while those of DNA synthesis utilized tritiated thymidine triphosphate. Incorporation of radioactivity was measured by counting glass fiber filters using liquid scintillation. Our data indicates that the trans-DDP is far

more potent than cis-DDP in inhibiting RNA transcription. Ninety percent inhibition was seen using 10 uM trans-DDP while less than 5% inhibition was seen using cis-DDP. The inhibitions of RNA transcription appear to be due to effects on the polymerase enzyme rather than to effects on the DNA template. DNA synthesis, however, appeared to be inhibited by effects on both the enzyme and the nucleic acid template. (no Refs)

18. HYDROXYUREA RESISTANT S49 CELLS WITH INCREASED M2 SUBUNIT OF RIBONUCLEOTIDE REDUCTASE (MEETING ABSTRACT).

Ullman B, Eriksson S, Jones T, Martin DW
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Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

Hydroxyurea is a cytotoxic agent which blocks DNA synthesis by inhibiting the enzyme ribonucleotide reductase. S49 cells isolated after several mo selection with increasing concentration of hydroxyurea showed a 20-fold increased resistance to the drug compared to wild-type cells. The ribonucleotide reductase activity in hydroxyurea resistant cells was equally sensitive to inhibition to hydroxyurea. However, when the activities of the two separated subunits of the enzyme (M1 and M2) were determined, subunit M2 activity was increased 10-fold while subunit M1 activity was threefold lower than in wild-type cells. The result can be interpreted as a selective amplification of one of the two subunits (M2) of ribonucleotide reductase leading to resistance to the growth-inhibiting effects of hydroxyurea. (2 Refs)

19. RIBONUCLEOSIDE DIPHOSPHATE REDUCTASE IN S49 MOUSE T-LYMPHOMA CELLS; CELL-CYCLE DEPENDENT VARIATION IN THE ACTIVITY OF ONE OF ITS SUBUNITS, PROTEIN M2 (MEETING ABSTRACT).

Eriksson S, Martin DW
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Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

The cell is dependent on the activity of ribonucleotide reductase for the produc tion of deoxyribonucleoside triphosphates necessary for DNA synthesis. This enzyme is composed of two nonidentical, loosely associated subunits--M1 and M2. We have measured the activity of the two subunits M1 and M2 separately in extracts from mouse S49 cells in different growth phases. Cells were treated with dibutyryl cyclic AMP which leads to the arrest of the cell cycle in the G1 phase. Exponentially growing cells were fractionated by centrifugal elutriation into S phase and G1 phase cells. With both methods a drastic decrease of ribonucleotide reductase activity was found in G1 cells. This effect was the consequence of decreased subunit M2 activity while subunit M1 activity was not changed during the cell cycle. (2 Refs)

20. PREFERENTIAL ASSOCIATION OF URACIL-DNA GLYCOSYLASE ACTIVITY WITH REPLICATING SV40 MINICHROMOSOMES.

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FEBS Lett; 133(1):89-91 1981

The preferential association of a major DNA repair enzyme, uracil-DNA glycosylase, with replicating SV40 minichromosomes is investigated. A nuclear extract containing SV40 minichromosomes, but free of cellular DNA and mitochondria was centrifuged to separate replicating and mature minichromosomes. Uracil-DNA glycosylase was tested by collecting fractions from the nuclear extract and incubating with an assay buffer and d3H uracil monophosphate (dUMP) containing DNA. The release of acid or ethanol soluble radioactivity was monitored. Cytochrome c oxidase was determined in aliquots of 100 ul from extracts or gradient fractions. To label both replicating and mature SV40 chromatin, SV40 infected CV-1 cells were incubated with 14C thymidine. Nuclear extracts were prepared as above. Uracil-DNA glycosylase activity was measured in each fraction. Results showed that uracil-DNA glycosylase cosediments with replicating chromatin; a smaller fraction cosedimented with mature chromatin. Thin layer chromatography of the ethanol soluble radioactivity released showed 90% comigrated with uracil. No cytochrome c oxidase activity cosedimented with SV40 minichromosomes. Apparently cosedimentation of uracil-DNA glycosylase with replicating chromatin is not due to contamination by mitochondria. The results suggest that uracil-DNA glycosylase is preferentially associated with replicating SV40 minichromosomes. This may indicate that misincorporation of dUMP is a more frequent event than cytosine deamination. (24 Refs)

MECHANISMS OF DNA REPAIR

21. ENZYMES INVOLVED IN THE REPAIR OF DAMAGED DNA.

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Arch Biochem Biophys; 211(2):511-522 1981

Enzymes responsible for the error-free repair of DNA damage induced by radiation and chemicals are reviewed. They include DNA glycosylases, photolyase, endonucleases, exonucleases, and DNA polymerases. Also discussed are repair due to metabolic alterations, mismatch base excision repair and reinsertion of nucleotides. (81 Refs)

22. CAPACITIES OF MOUSE EMBRYO CELLS FOR INDUCTION OF UNSCHEDULED DNA SYNTHESIS BY 4-NITROQUINOLINE 1-OXIDE IN PRIMARY CULTURE SYSTEMS.

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JNCI; 67(5):1063-1069 1981

Because 4-nitroquinoline 1-oxide (4-NQO) selectively induces lung tumors in offspring of ICR/Jcl inbred mice, unscheduled DNA synthesis (UDS) induced by 4-NQO was investigated with the use of confluent primary cultures of cells from six different organs of ICR/Jcl mouse embryos. The UDS level of lung-derived cells (lung cells), as measured with hydroxyurea-resistant incorporation of [3H]thymidine into cellular DNA in the presence of 4-NQO, was at least threefold lower than levels in cells from skin, intestine, kidney, liver, and brain, when compared at equitoxic doses of 4-NQO or equimolar binding of 4-NQO to DNA. Autoradiographic studies also revealed that the percentage of UDS-positive nuclei in lung cells was about one-fifth of other cell types. Cytotoxic examinations

showed that the lung cells were the most sensitive to the lethal action of 4-NQO. These results suggest that the capacity of cells from the target organ to repair 4-NQO damage is intrinsically much lower than the capacity of cells from nontarget organs. (Author abstract) (30 Refs)

23. DNA REPAIR CAPABILITIES OF VARIOUS MOUSE SPERMATOGENIC STAGES AFTER TREATMENT WITH ENU AND MNU (MEETING ABSTRACT).

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Eleventh Annual Meeting of the European Environmental Mutagen Society, July 6-11, 1981, Budapest, Hungary The Society; 1981

ENU (ethylnitrosourea) has been shown to be the most potent mutagen in the mouse with regard to the induction of specific locus mutations in spermatogonia. On the other hand, methylnitrosourea (MNU), a closely related compound is totally ineffective in the same system. MNU methylates the DNA of germ cells and is highly effective in inducing unscheduled DNA synthesis in mouse spermatids. Therefore the lack of mutagenic effect by MNU in spermatogonia is not due to a failure to reach the genetically significant targets in the testis. (101 x C3H)F1 male mice were injected with ENU or MNU dissolved in Sorensen buffer at pH 6.0. All mice received injections of [3H] dThd into the testes. The activity injected was 36uCi per testes. The activity injected was 36uCi per testis. The unscheduled incorporation of [3H]-thymidine into the DNA of germ cells was measured by liquid scintillation counting techniques and by autoradiography. The results obtained so far show that meiotic through round spermatid stages are capable of repairing DNA damage induced by ENU. However the repair response induced by ENU in pachytene and early spermatid stages is much smaller than that induced by MNU at equimolar concentrations. (3 Refs)

24. EFFECT OF METALS ON MUTAGENESIS AND DNA REPAIR.

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Environ Health Perspect; 40:189-195 1981

A review is presented of mechanisms of mutagenesis, effects on DNA repair leading to comutagenesis, and possible reasons why carcinogenic metals are not mutagenic in microbial systems. A classification of mutagenesis mechanisms is based on the possible modes of action of metals: misreplication due to altered DNA or decreased fidelity of DNA polymerase, inhibition of proofreading, and mutagenesis via error-prone DNA repair. Excision repair, mismatch repair, and two other alternative pathways (not involving cleavage of the phosphodiester bond and subsequent repair replication characteristic of excision repair) are discussed with respect to effects on DNA repair leading to comutagenesis. Bacteria have an inducible, error-prone DNA repair system (SOS repair) whose activity results in mutagenesis. In one of the best studied examples of metal mutagenesis, chromate, little evidence exists for involvement of the SOS system. Arsenite exemplifies metals that may act as comutagens by inhibiting repair of damage to DNA caused by another agent. Comutagens would not be detected by standard screening methods. (48 Refs)

25. BLOCKING BY THE CARCINOGEN, L-ETHIONINE, OF SOS FUNCTIONS IN A TIF-1 MUTANT OF ESCHERICHIA COLI B/R.

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Cancer Res; 41(11,part1):4382-4385 1981

In Escherichia coli, DNA damage by carcinogenic agents results in the coordinate expression of a diversity of functions (SOS functions), many of which are thermally inducible without any damage to DNA in a tif-1 mutant. These include prophage induction, filamentous growth, and an *error-prone DNA repair* activity, which is responsible for UV-induced mutagenesis. Ethionine causes hepatic carcinoma in rats after prolonged feeding but is not a mutagen in the Ames test. The present study shows that 10 mM ethionine prevents the thermal induction of lambda-prophage in a tif-1 derivative of E. coli. The enhancement of mutation, which normally occurs at high temperature after a low dose of UV light, is also blocked by ethionine. Ethionine does not block, to any appreciable extent, the incorporation of radioactive precursors into RNA, DNA, or protein. (Author abstract) (30 Refs)

26. REPAIR OF O6-METHYLGUANINE IN BACTERIAL DNA: MECHANISMS OF MUTATION AVOIDANCE AF TER ALKYLATION (MEETING ABSTRACT).

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Eleventh Annual Meeting of the European Environmental Mutagen Society, July 6-11, 1981, Budapest, Hungary The Society; 1981

O6-methylguanine is one of a number of modified bases produced in DNA by alkylating mutagens. It has been implicated in mutagenesis and shown to miscode when present in template DNA in assays in vitro. Escherichia coli avoid the mutagenic and lethal effects of alkylating agents via a phenomenon known as the adaptive response to alkylating agents. At least part of this response involves the repair of O6-methylguanine in DNA. The protein responsible for this repair is induced by the treatment with alkylating agents and acts by removing the methyl group from the 06-position of the alkylated base thus restoring the original guanine. No excision and resynthesis of DNA is involved in this repair. In addition, we have evidence that a second pathway may act on O6-methylguanine in bacterial DNA. This process - which is probably constitutive - requires intact dam and mutL genes. In experiments carried out under conditions in which the adaptive response is not induced, we have compared the sensitivity to killing by different alkylating agents of mutL, dam, and mutL dam strains. Our results are consistent with the idea that a mis-match correction process operates on replicated O6-methylguanine in wild-type cells and that this process is impaired in dam and mutL dam strains. Several predictions about the interaction of the adaptive response with this process have been fulfilled. (no Refs)

27. ASSESSING CONSEQUENCES OF MARINE POLLUTION BY HYDROCARBONS USING SPONGES AS MODEL ORGANISMS.

Zahn RK, Zahn G, Muller WE, Kurelec B, Rijavec M, Batel R, Given R

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Sci Total Environ; 20(2):147-169 1981

Mutagenic activity in marine sponges (mainly Tethya lyncurium from the Northern Adriatic and from Catalina Island off

Los Angeles, California) due to polycyclic aromatic hydrocarbon (PAH) pollution was investigated using radiolabeled benzo(a)pyrene (BaP) as a model pollutant. Tethya was exposed to Diesel 2 oil pollution at a level of 1-6 ppm. The amount of label converted into any one of three polyamines changed significantly: in controls, 30% ornithine decarboxylated were found as amines, while under conditions of oil pollution 80% were found as amines. With the Ames test the pollutant level was shown to be 3+ (equivalent to 0.7 ppb BaP). Ornithine decarboxylase activity increased slightly during the first 16 days of exposure, but had increased 10x by day 24. An increase in DNA-dependent RNA polymerase activity was seen with exposure to 1 ppm Diesel 2 for 1-2 days; the increase was only significant 1-2 days after exposure. Mixed f unction oxidase (MFO) activity could not be detected. Significant coupling of BaP derivatives to protein, DNA, and RNA was observed; the largest amount disappeared in the protein fraction. When illumination of 5 x 10(4) lux was omitted, the coupling of BaP derivatives to the DNA fraction dropped to 1/74x the previous values or less. Increased binding of BaP derivatives to DNA in the presence of light was significant between 0.001 and 31 ppb. However, when activated microsomal fractions from fish that had been exposed to high levels of PAH were added, dark incorporation rose to significant levels which could be decreased by the addition of the MFO inhibitor 7,8-benzoflavone. Possible absence of ***DNA repair*** in the sponges is discussed (52 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

28. INSERTION OF NEW GENES INTO BONE MARROW CELLS OF MICE.

Cline MJ, Stang HD, Mercola K, Salser W
Dept. Medicine, Univ. California, Center for Health Sciences, Los Angeles, CA, 90024
Haematol Blood Transfus; 26:167-170 1981

Utilizing existing techniques for inserting new genes into bone marrow stem cells of living mice and for favoring the proliferation of transformed stem cells carrying the new genes, experiments were carried out to insert the mouse dihydrofolate reductase (DHFR) genes resistant to methotrexate (MTX) and the herpes simplex virus thymidine kinase (HSV-tk) genes into mouse stem cells. The insertion of the MTX-resistant DHFR genes permitted the use of MTX challenge to favor the proliferation of transformed cells. The simultaneous insertion of HSV-tk genes permits the transformed cells to grow in the presence of MTX by utilizing the salvage pathway of DNA synthesis more efficiently. Karyotype analysis confirmed the existence of transformed stem cells. Efficient gene expression was obtained for both of the inserted gene sequences. This technique may have direct clinical application in treating hemoglobinopathies and hematopoietic malignancies and in reducing the hematopoietic toxicity of anticancer drugs. (11 Refs)

29. DNA SEQUENCE OF A MAJOR HUMAN LEUKOCYTE INTERFERON GENE.

Lawn RM, Gross M, Houck CM, Franke AE, Gray PV, Goeddel DV
Dept. Molecular Biology, Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, CA, 94080
Proc Natl Acad Sci USA; 78(9,part2):5435-5439 1981

The gene for human leukocyte interferon alpha2 (designated either LeIF or HuIFN-alpha2) has been isolated from a human genome library. The DNA sequence of this gene demonstrates that it lacks introns. The 3' noncoding sequences of the IFN-alpha2 gene correspond to two types of IFN-alpha2 cDNA clones we have isolated that have alternate sites of polyadenylylation. A comparison of seven human IFN-alpha sequences shows that they are homologous in the 5' flanking region and contain identical 'TATA box' s equences. The recombinant lambda clone containing the IFN-alpha2 gene also contains two copies of the 'Alu family' repeat sequence. (Author abstract) (38 Refs)

30. STRUCTURE OF A CHROMOSOMAL GENE FOR HUMAN INTERFERON BETA.

Ohno S, Taniguchi T
Dept. Biochemistry, Cancer Inst., Japanese Foundation for Cancer Research, Toshima-ku, Tokyo 170, Japan
Proc Natl Acad Sci USA; 78(9,part2):5305-5309 1981

We have cloned and analyzed a chromosomal DNA segment containing the human interferon beta1 gene from a human gene library. The nucleotide sequence of the protein-coding and the noncoding regions of the chromosomal gene was identical to the cDNA sequence reported previously. In the region upstream from the putative transcription initiation site, significant nucleotide sequence homology was observed between interferon beta1 and alpha1 genes. This region thus may play a role in expression of the interferon genes. From the sequence data and the result of nuclease S1 mapping experiments, we conclude that, like the interferon alpha1 gene, the interferon beta1 gene is devoid of intervening sequences. (Author abstract) (40 Refs)

31. ORGANIZATION OF THE METHOTREXATE RESISTANT MOUSE L5178YR DIHYDROFOLATE REDUCTASE GENE AND TRANSFORMATION OF HUMAN HCT-8 CELLS BY THIS GENE.

Scheer DI, Srimatkandada S, Kamen S, Dube S, Bertino JR
(c/o Bertino), Yale Univ. Sch. Medicine, 333 Cedar St., New Haven, CT, 06510
Haematol Blood Transfus; 26:171-174 1981

To investigate the role of gene amplification in the development of cell resistance to methotrexate (MTX) and the regulation of the dihydrofolate reductase (DHFR) gene, an analysis of the DNA sequence organization of the DHFR gene was carried out. Southern blot hybridization of enzymatically cleaved DNA from the mouse lymphoblastoid line L5178YR suggests that the DHFR gene is interrupted by at least five intervening sequences and is at least 45 kilobase pairs in size. When this DNA was used to transform the human colon carcinoma cell line HCT-8, the transformants were stably resistant to MTX and contained 10-fold elevations of DHFR compared with parental HCT-8 cells. Southern blot hybridization analysis comparing the transformed DHFR gene with the human DHFR gene supports the conclusion that the mouse DHFR gene is stably integrated into the human cell line. Definitive structural data on the transformed sequence will have to be ob-

tained by characterizing fragments of the gene obtained by molecular cloning. (12 Refs)

32. CELLULAR HOMOLOGUE (C-SRC) OF THE TRANSFORMING GENE OF ROUS SARCOMA VIRUS: ISOLATION, MAPPING, AND TRANSCRIPTIONAL ANALYSIS OF C-SRC AND FLANKING REGIONS.

Parker RC, Varmus HE, Bishop JM

Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143

Proc Natl Acad Sci USA; 78(9,part2):5842-5846 1981

The tumorigenic properties of Rous sarcoma virus are attributable to a 60,000-dalton protein, pp60v-src, encoded by a single viral gene, v-src, A homologous gene, c-src, that contains the information for a 60,000-dalton protein, pp60c-src, has been identified in all tested vertebrate cells. By screening a recombinant DNA library representative of the chicken genome, we isolated two overlapping DNA fragments that contain more than 30 kilobases (kb) of DNA spanning the coding sequences for pp60c-src. This 30-kb region is devoid of moderately or highly repeated sequences and shares homology with the entire viral gene and noncoding sequences 5' of v-src. Although v-src has an uninterrupted coding sequence, c-src is interrupted by a minimum of seven intervening sequences. At least two polyadenylylated RNAs seem to be encoded within the DNA we have isolated. The larger RNA, approx 3.9 kb, is the presumptive c-src mRNA; the smaller transcript of about 2 kb hybridizes to DNA sequences several kb from the 3' end of the v-src/c-src homology area. (Author abstract) (28 Refs)

33. SEQUENCE DIVERGENCE OF NUCLEUS-CONFINED POLYADENYLATED RIBONUCLEIC ACIDS IN FRIEND ERYTHROLEUKEMIC CELLS.

Balmain A, Sproul A, Birnie GD

Wolfson Lab. Molecular Pathology, Beatson Inst. Cancer Res., Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, Scotland

Biochemistry; 20(21):6257-6264 1981

For determination of whether any potential protein-coding RNA sequences are confined to the nucleus in Friend cells, a comparison has been made of the sequence divergence of polyadenylated RNA molecules which are either nucleus confined or transported to the cytoplasm. Both unique DNA and cDNA probes enriched in such sequences were reacted with a large excess of mouse or rat DNA. The extent of hybridization and the thermal stability of hybrids formed under various stringency conditions were used as a measure of the amount of sequence divergence which had taken place. The results confirmed that messenger sequences are relatively conserved during evolution. Furthermore, experiments involving both unique DNA probes representative of the full length of nucleus-confined RNA molecules and cDNA probes complementary to their poly(A)-adjacent fragments indicated that the polyadenylated nucleus-confined sequences have, on av, diverged during evolution much more than mRNA sequences. The vast majority of these sequences therefore probably do not represent potential mRNAs which are confined to the nucleus and subject to posttranscriptional control in a manner similar to those which have been demonstrated in sea urchins. (Author abstract) (40 Refs)

34. SYNTHESIS OF STABLE UNSPLICED MRNA FROM AN INTRONLESS SIMIAN VIRUS 40-RAT PREPROINSULIN GENE RECOMBINANT.

Gruss P, Efstratiadis A, Karathanasis S, Konig M, Khoury G

Lab. Molecular Virology, NCI, Bethesda, MD, 20205

Proc Natl Acad Sci USA; 78(10):6091-6095 1981

We constructed a recombinant DNA molecule carrying the coding region and the 3' flanking sequences of the rat preproinsulin I gene, replacing the late region of a simian virus 40 (SV40) viral vector. The sequences making up the single intron of the preproinsulin I gene, located within the nontranslated leader region, as well as all of the 5' flanking sequences, have been deleted. The sequences remaining within the late transcriptional unit of SV40 do not retain a functional intron. This 'intronless' mutant nevertheless directs the synthesis of substantial quantities of unspliced stable hybrid transcripts that are translated into polypeptides with insulin immunoreactivity. Two novel 5' termini, not used in wild-type SV40 transcripts, have been detected in the majority of the hybrid RNA molecules. These are located at analogous positions in both of the late SV40 72-base-pair repeats. (Author abstract) (34 Refs)

35. DNA METHYLATION AND METHYLASE LEVELS IN NORMAL AND MALIGNANT MOUSE HEPATIC TISSUES.

Lapeyre JN, Walker MS, Becker FF

Dept. Pathology, Section Experimental Pathology, M.D. Anderson Hosp. and Tumor Inst., 6723 Bertner Ave., Houston, TX, 77030

Carcinogenesis; 2(9):873-878 1981

DNA methylation levels (expressed as the percentage of 5-methylcytosine relative to total cytosines) were measured in normal mouse liver, primary hepatocellular carcinomas (PHCs) in male C3H mice, PHCs induced in male C7Bl/6N mice by chlordane, N-acetylaminofluorene, or 3'methyl-4-dimethylaminoazobenzene, and spontaneous and chemically induced PHCs in male (C7BL/6H x C3H/HeN)F1 (B6C3) mice. All tumors demonstrated an undermethylation of their genomes, compared with normal livers of age-matched controls. The undermethylation was not due to inactivation of or deficiency in DNA methylase activity, since enzyme levels in tumor nuclei were higher than background levels. The possibility that aberrations in DNA methylation are involved in the transformation of normal differentiated cells to neoplastic cells is discussed. (57 Refs)

OTHER RELATED STUDIES

36. VINCRISTINE-RESISTANT HUMAN NEUROBLASTOMA CELLS HAVE DOUBLE MINUTES (DMS) AND HOMOGENEOUSLY STAINING REGIONS (HSRS) (MEETING ABSTRACT).

Meyers MB, Spengler BA, Biedler JL

Sloan-Kettering Institute, 145 Boston Post Road, Rye, NY, 10580

In Vitro; 17(3):221 1981

HSRs of metaphase chromosomes are sites of amplified dihydrofolate reductase-encoding genes in rodent cells highly resistant to antifolates. We recently reported the finding of an HSR in vincristine-resistant Chinese hamster lung cells. These cells oversynthesize a low mol wt protein, pI 5.7. Two vincristine-resistant human neuroblastoma cell strains have now been de-

veloped and studied. SH-SY5Y/VCR, a s ubclone of the SK-N-SH line, and MC-IXC/VCR, a subclone of the SK-N-MC human neuroblastoma line, show high levels of resistance to vincristine. Karyotype analysis revealed that 100% of SH-SY5Y/VCR cells have 10-100 very small DMs; neither DMs nor HSRs have ever been found in drug-sensitive cells of the SK-N-SH line or its clones. MC-IXC/VCR cells, on the other hand, consistently contain HSR-like segments on chromosomes 19p and 22q in contrast to a shorter HSR-like segment on 22q only, in 23% of control cells. As demonstrated by 2D gel analysis, the vincristine-resistant human lines contain increased amounts of a low mol wt acidic protein. Whether or not this protein is the product of an amplified gene, the cytological finding suggest strongly that DNA sequence amplification has occurred in the vincristine-resistant human cells. (no Refs)

37. CHEMICAL SYNTHESIS OF RADIOLABELED BLEOMYCIN A2 AND ITS BINDING TO DNA.

Roy SN, Orr GA, Brewer FC, Horwitz SB

Dept. Molecular Pharmacology, Albert Einstein Coll. Medicine, Bronx, NY, 10461

Cancer Res; 41(11,part1):4471-4477 1981

A method for the preparation of biologically active [3H]- and [13C]bleomycin A2 is described. Demethyl Cu(II):bleomycin A2, isolated after pyrolysis of Cu(II):bleomycin A2, was methylated with either [3H]- or [13C]methyl iodide, which resulted in Cu(II):bleomycin A2 labeled in the dimethylsulfonium moiety. Copper was removed by treatment with dithizone in chloroform, and structures were verified by thin-layer chromatography and 1H and 13C magnetic resonance spectroscopy. Copper-free [3H]- and [13C]bleomycin A2 are active in the degradation of DNA in vitro. Gel exclusion chromatography and equilibrium dialysis were used to determine the apparent equilibrium constants for binding of [3H]bleomycin A2 and Cu(II):[3H]bleomycin A2 to calf thymus DNA, noncovalently associated polydeoxyguanylate:polydeoxythymidylate, and noncovalently associated polydeoxyadenylate:polydeoxythymidylate. In 2.5 mM sodium phosphate buffer, pH 7.0, binding data obtained by gel filtration with calf thymus DNA reveal an apparent equilibrium constant for [3H]bleomycin A2 of 5.7 x 10(5)/mol and for Cu(II):[3H]bleomycin A2 of 3.9 x 10(5)/mol. One molecule of [3H]bleomycin A2 binds for every 3.7 base pairs in DNA, and one molecule of Cu(II):[3H]bleomycin A2 binds for every 2.8 base pairs in DNA. Analysis of binding data with calf thymus DNA, noncovalently associated polydeoxyguanylate:polydeoxycytidylate, and noncovalently associated polydeoxyadenylate:polydeoxythymidylate obtained by equilibrium dialysis reveals, in each instance, two types of binding sites for both the copper and metal-free form of the antibiotic. For those sites in calf thymus DNA with tighter binding affinity, the apparent equilibrium constant for [3H]bleomycin A2 was 6.8 x 10(5)/mol and for t he Cu(II):[3H]bleomycin A2 complex, 4.4 x 10(5)/mol. As seen with calf thymus DNA, the affinity of [3H]bleomycin A2 is slightly greater than that of Cu(II):[3H]bleomycin A2 for the synthetic DNAs, although more of the copper form of the drug binds to these polymers. (Author abstract) (35 Refs)

38. ADDUCTS FROM THE REACTION OF O,O'-DIACETYL OR O-ACETYL DERIVATIVES OF THE CARCINOGEN 4-HYDROXYAMINOQUINOLINE 1-OXIDE WITH PURINE NUCLEOSIDES.

Bailleul B, Galiegue S, Loucheux-Lefebvre M-H
(c/o Loucheux-Lefebvre) Institut Recherches le Cancer de Lille et Unite, 124 de l'INSERM, BP 311, 59020 Lille Cedex, France

Cancer Res; 41(11,part1):4559-4565 1981

The diacetyl derivative of 4-hydroxyaminoquinoline 1-oxide (4-HAQO), the proximate carcinogen of 4-nitroquinoline 1-oxide, was reacted in vitro with purine nucleosides to give five adducts (3 with guanosine and 2 with adenosine). The same nucleoside modifications were also obtained with a monoacetyl derivative of 4-HAQO which is probably 4-acetoxy-aminoquinoline 1-oxide. The structure of the major adduct (the so-called dG III) was identified as N-(deoxyguanosine-C8-yl)-4-aminoquinoline 1-oxide. The isolation of this adduct from the 4-HAQO-modified DNA in vivo provides strong support for the hypothesis that the acetyl derivatives of 4-HAQO constitute a good model for the ultimate carcinogen. (Author abstract) (21 Refs)

39. MUTAGENICITY OF THE REACTION PRODUCTS OF L-ASCORBIC ACID AND L-TRYPTOPHAN.

Kanamori H, Morimoto K, Kinae N, Tomita I
Hiroshima Prefectural Institute of Public Health, Uzina-Kanda, Hiroshima 734, Japan

Yakugaku Zasshi; 101(7):596-604 1981

Glycine, dl-phenylalanine, l-tryptophan, and ammonia were allowed to react with l-ascorbic acid and d-glucose, in a 0.1M phosphate buffer (pH 7.0) at 37 C for 2 mo; the reaction mixtures were analyzed structurally and were tested for mutagenicity to Salmonella typhimurium TA 98 and TA 100 and to Bacillus subtilis (Ames test). Except for the mixture of l-ascorbic acid and ammonia, DNA-damaging effects were seen for reaction mixtures of l-ascorbic acid, but none were observed for reaction mixtures of d-glucose. Reaction mixtures of l-tryptophan and l-ascorbic acid were found to be DNA-damaging at day 8 after mixing, and the activity of the reaction mixture increased by mo 2, but DNA-damaging activity of this reaction mixture decreased after 2 mo. Following extraction of the reaction products of l-tryptophan and l-ascorbic acid with benzene and ethyl acetate and purification with silica-gel column chromatography and thin layer chromatography, mutagenic activity against S typhi TA 100 was observed for one fraction containing 1-(2-furyl)-9H-pyrido-[3,4-b]-indole with or without S-9 mixture and for 1-(2-furyl)-9H-pyrido[3,4-b]-indole carboxylic acid in the absence of the S-9 mixture. (15 Refs) (In JPN)

40. INTERACTION OF 1-(5-PHOSPHO-BETA-D-ARABINOFURANOSYL)-5-SUBSTITUTED URACILS WITH THYMIDYLATE SYNTHETASE: MECHANISM-BASED INHIBITION BY 1-(5-PHOSPHO-BETA-ARABINOSYL)-5-FLUOROURACIL.

Nakayama C, Wataya Y, Santi DV, Saneyoshi M, Ueda T
Faculty Pharmaceutical Sciences, Hokkaido Univ., Sapporo 060, Japan

J Med Chem; 24(10):1161-1165 1981

A number of 1-(5-phospho-beta-D-arabinosyl)-5-substituted-uracils (ara-UMP's) have been examined as inhibitors of dTMP synthetase. As reversible inhibitors, all were substantially less potent than their 2'-deoxyribosyl counterparts. In the presence of 5,10-methylenetetrahydrofolate (CH2-H4folate), ara-FUMP caused a first-order, time-dependent inactivation of the

enzyme. At 0 C, kinetic studies indicated a reversible Kd of 3.6 uM for the ara-FUMP-CH2-H4folate complex, and kappa = 0.22 min-1 for the subsequent inactivation. Spectral studies of the complex and its behavior toward protein denaturants demonstrate that its structure and stoichiometry are directly analogous to those which have previously been described for FdUMP. The significance of this finding with regard to prodrugs of ara-FU and the potential of ara-FU as a chemotherapeutic agent are discussed. (Author abstract) (33 Refs)

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MECHANISMS OF DNA REPLICATION

1. STRUCTURE OF CHROMATIN AT DEOXYRIBONUCLEIC ACID REPLICATION FORKS: PRENUCLEOSOMAL DEOXYRIBONUCLEIC ACID IS RAPIDLY EXCISED FROM REPLICATING SIMIAN VIRUS 40 CHROMSOMES BY MICROCOCCAL NUCLEASE.

Cusick ME, Herman TM, DePamphilis ML, Wassarman PM
Dept. Biological Chemistry, Harvard Medical Sch., Boston, MA, 02115
Biochemistry; 20(23):6648-6658 1981

Replicating simian virus 40 (SV40) chromsomes were found to be similar to other eukaryotic chromosomes in that the rate and extent of micrococcal nuclease (MNase) digestion were

HE 20.3172/2:

greater with replicating than with nonreplicating mature SV40 chromatin. MNase digestion of replicating SV40 chromsomes, pulse labeled in either intact cells or nuclear extracts, resulted in the rapid release of nascent DNA a s essentially bare fragments of duplex DNA (3-7 S) that had an av length of 120 base pairs and were degraded during the course of the reaction. The small nascent DNA fragments released from replicating chromosomes represented prenucleosomal DNA (PN-DNA) from the region of replication forks that encompasses the actual sites of DNA synthesis and includes Okazaki fragments. Predigestion of replicating SV40 chromosomes with both Escherichia coli exonuclease III (3'-5') and bacteriophage T7 gene 6 exonuclease (5'-3') resulted in complete degradation of PN-DNA. This result, together with the observation that isolated PN-DNA annealed equally well to both strands of SV40 restriction fragments, demonstrated that PN-DNA originates from both sides of replication forks. Over 90% of isolated Okazaki fragments annealed only to the retrograde DNA template. The characteristics of isolated PN-DNA were assessed by examining its sensitivity to MNase and single strand specific S1 endonuclease, sedimentation behavior before and after deproteinization, buoyant density in CsCl after formaldehyde treatment, and size on agarose gels. In addition, it was observed that MNase digestion of purified SV40 DNA also resulted in the release of a transient intermediate similar in size to PN-DNA, indicating that a DNA-protein complex is not required to account for the appearance of PN-DNA. These and other data provide a model of replicating chromosomes in which DNA synthesis occurs on a region of replication forks that is free of nucleosomes and is designated as prenucleosomal DNA. (57 Refs)

EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

2. A WASHABLE MACROMOLECULE FROM FV2RR MARROW NEGATIVELY REGULATES DNA SYNTHESIS IN ERYTHROPOIETIC PROGENITOR CELLS BFU-E.

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Cell; 26(2,part2):233-244 1981

The proportion of BFU-E normally engaged in DNA synthesis is low in adult B6 (C57BL/6) mice of genotype Fv2rr (resistant to Friend erythroleukemia virus), as shown by 3H-thymidine or hydroxyurea 'cell suicide' experiments in vivo and in vitro. When bone marrow cells from these mice were subjected to a single wash in alpha medium, the proportion of BFU-E synthesizing DNA dramatically rose to levels as high as those normally seen among the BFU-E of congenic B6.S mice of genotype Fv2ss (sensitive to Friend erythroleukemia virus). Washing Fv2rr marrow cells did not significantly affect the proportion of CFU-S, CFU-nm (CFU-C) or CFU-E engaged in DNA synthesis. An activity responsible for keeping low the proportion of BFU-E in DNA synthesis was recovered in supernatants of Fv2rr (but not Fv2ss) bone marrow cells; its effect could be demonstrated on the BFU-E of either Fv2rr or Bf2ss washed bone marrow cells. This activity was n ontoxic to the BFU-E, rapidly reversible and effective at, but not far below, the concentrations normally found in adult Fv2rr marrow. It was stable to 56 C for 30 min, was nondialyzable, appeared in the void vol on G-25 Sephadex gel filtration and could be filtered through membranes that permitted passage of particles of less than 100,000 but not less than 50,000 daltons. Thus the Fv2 locus (or a locus closely linked to it) appears to act not in the BFU-E itself, but elsewhere, to control the amount or activity of a macromolecular negative regulator to which the BFU-E population responds by a reduction in the proportion synthesizing DNA. This study reveals the existence of a negative growth control mechanism for early erythropoietic progenitor cells, which is apparently physiological in nature and under strict genetic control. (Author abstract) (24 Refs)

3. ADENOSINE 5'-TRIPHOSPHATE- (ATP) MEDIATED STIMULATION AND SUPPRESSION OF DNA SYNTHESIS IN LYMPHOID CELLS. I. CHARACTERIZATION OF ATP RESPONSIVE CELLS IN MOUSE LYMPHOID ORGANS.

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J Immunol; 127(5):1834-1838 1981

The effects of various nucleotides and nucleosides on DNA synthesis of mouse lymphocyte populations were studied. Significant stimulation of DNA synthesis was observed in the cells from bone marrow or the thymus in the presence of ATP. In contrast, the DNA synthesis of the cells from spleen, lymph nodes, and peripheral blood was markedly inhibited by ATP. Guanosine 5'-triphosphate had nonspecific stimulatory effects on the DNA synthesis of various lymphoid cells, whereas cytidine triphosphate had no effect. When the thymocytes or bone marrow cells were separated by 1 x g velocity sedimentation, a distinct cell population was identified as being responsible for the ATP-mediated stimulation of DNA synthesis. Further characterization of ATP-responsive cells revealed that the highest concentration of terminal deoxynucleotidyl transferase, a marker enzyme for precursor T cells, was present in these cells. ATP-mediated stimulation of DNA synthesis may, therefore, serve as a specific marker restricted to a certain population of differentiating T cells. (Author abstract) (32 Refs)

4. CYTOKINETIC AND BIOCHEMICAL EFFECTS OF 5-IMINODAUNORUBICIN IN HUMAN COLON CARCINOMA IN CULTURE.

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Cancer Res; 42(1):117-121 1982

The semisynthetic anthracycline, 5-iminodaunorubicin (IM), was investigated to see whether modification of the benzoquinone moiety to produce a drug with low free radical potential would alter the cytotoxic and biochemical characteristics of this drug in comparison to Adriamycin (ADR), an a gent with high free radical potential. Cell viability was measured in human colon carcinoma (HT-29) cells by soft-agar cloning. Upon exposure of either log-phase or early plateau-phase cells for 2 hr to IM or ADR, a threshold exponential cell lethality curve was obtained. Prolonging drug exposure to 24 hr produced an exponential decline in cell survival and a marked reduction in viability of both log-phase and early plateau-phase cells. Inhibition of DNA synthesis in log-phase cells after 2 and 24 hr of exposure to IM and ADR paralleled the increased cell lethality produced by the drugs. In contrast, total RNA synthesis was not inhibited by IM, whereas ADR impaired both RNA and *DNA synthesis.* Nuclear rRNA synthesis was not significantly inhibited following 24 hr of exposure to 10(-7) M ADR or IM but was inhibited by 85 and 35% at 10(-6) M ADR or IM, respectively. The affinity of IM and ADR for

HT-29 DNA was measured in vitro by displacement of acridine orange binding and was found to be similar for both analogs. These studies suggest that the cytotoxicity of IM and ADR results from the interactions of these drugs with DNA. (Author abstract) (20 Refs)

5. ABNORMAL REGULATION OF DNA REPLICATION AND INCREASED LETHALITY IN ATAXIA TELANGIECTASIA CELLS EXPOSED TO CARCINOGENIC AGENTS.

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Cancer Res; 42(1):335-341 1982

The effect of different carcinogenic agents on the rate of semiconservative DNA replication in normal human and ataxia telangiectasia (AT) cells was investigated. The rate of DNA synthesis in all AT cell strains tested was depressed to a significantly lesser extent than in normal cells after exposure to x-rays under oxia or hypoxia or to bleomycin, agents to which AT cells are hypersensitive. In contrast, inhibition of DNA replication in normal human and AT cells was similar after treatment with some DNA-methylating agents or mitomycin C. Colony-forming ability of AT cells treated with these agents was not different from normal cells. Treatment with 4-nitroquinoline 1-oxide elicited a variable response in both AT and normal cell strains. In some strains, including those shown to be hypersensitive to the drug by other workers, the inhibition of DNA synthesis was more pronounced than in other cell strains, but no significant difference between AT and normal cells could be detected. The rejoining of DNA strand breaks induced by x-rays, measured by DNA elution techniques, occurred within 2 hr after treatment and could not be correlated with the difference in DNA synthesis inhibition in AT and normal cells. After low doses of x-rays, AT cells rejoined single-strand breaks slightly more slowly than did normal cells. The rate of DNA replication in x-irradiated AT and normal cells was not affected by nicotinamide, an inhibitor of poly(adenosine diphosphate ribose) synthesis. These data indicate that the diminished inhibition of DNA replication in carcinogen-treated AT cells (a) is a general characteristic of all AT cell strains, (b) correlates with AT cellular hypersensitivity, (c) is not directly caused by the bulk of the DNA strand breaks produced by carcinogenic agents, and (d) is not based on differences in the induction of poly(adenosine diphosphate ribose) synthesis between x-irradiated AT and normal cells. (Author abstract) (47 Refs)

6. RADIORESISTANT DNA SYNTHESIS: AN INTRINSIC FEATURE OF ATAXIA TELANGIECTASIA.

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Lab. Radiobiology, Univ. California, San Francisco, CA, 94143
Mutat Res; 84(1):183-190 1981

Radioresistance of DNA synthesis (DNAS) was compared in normal human diploid cells of cell line HS27 and six strains derived from patients with ataxia telangiectasia (AT). Three of the AT cell lines had previously been classified as proficient in repair replication after gamma-irradiation. Inhibition of DNAS was measured by incorporation of 14C-thymidine. Cells of normal and AT lines were exposed to 0-5,000 rads of x-rays. To determine the rate of DNAS at various times after irradiation, cultures were pulse-labeled at various times after irradiation. DNAS was strongly inhibited by up to 1,000 rads in HS27, but not in AT cells. DNAS was inhibited to a lesser extent between 1,000 and 5,000 rads in both HS27 and AT cells. In HS27 cells DNAS was reduced to approx 35% of control values for 1 hr, increasing to 60% from 2 to 4 hr after irradiation. In AT cells DNAS was reduced to 75-80% of control values 90 min after irradiation and remained at that level after 4 hr. Inhibition of replicon initiation was the primary factor in radioresistance of AT cells. Chain elongation was also more radioresistant in AT cells than in HS27 cells. Up to 2,000 rads inhibition of DNAS in AT cells was due almost entirely to inhibition of replicon initiation and not of chain elongation. (17 Refs)

7. EFFECT OF 1,2-DIMETHYLHYDRAZINE AND DIETHYLNITROSAMINE ON CELL REPLICATION AND UNSCHEDULED DNA SYNTHESIS IN TARGET AND NONTARGET CELL POPULATIONS IN RAT LIVER FOLLOWING CHRONIC ADMINISTRATION.

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Cancer Res; 42(1):89-92 1982

Marked differences in the rates of de novo DNA synthesis and unscheduled DNA synthesis were observed in hepatocytes and nonparenchymal cells (NPC) isolated from Fischer 344 rats following in vivo exposure to 1,2-dimethylhydrazine (SDMH) or diethylnitrosamine (DEN) for 1.5, 4, 8, 16, and 28 days. Liver cells were labeled in vivo with bromodeoxyuridine and [3H]thymidine, following which, hepat ocytes and NPC were separated by elutriation centrifugation. Purified DNA was then separated into fully replicated and nonreplicated DNA by ultracentrifugation in CsCl gradients. Following exposure to SDMH, the rate of de novo DNA replication in NPC was greatly increased over both control NPC and the corresponding hepatocytes for the entire time course. Unscheduled DNA synthesis was only observed in hepatocytes after exposure to SDMH. Following exposure to DEN, both cell populations exhibited changes in de novo DNA synthesis that were directly proportional to one another except at 1.5 days. Increases occurred first in hepatocytes at 1.5 days but were delayed in NPC until 4 days. Hepatocyte replication returned to control values by 28 days. Unscheduled DNA synthesis was much lower following exposure to DEN than SDMH. Therefore, SDMH, which induces hemangioendotheliomas of the liver at the dose given, induces a marked increase in cell replication in the target cell population and unscheduled DNA synthesis in the resistant cell population. DEN, which induces hepatocellular carcinoma, moderately stimulates cell replication in both liver cell populations and induces little unscheduled DNA synthesis in either population. (Author abstract) (19 Refs)

8. 1-BETA-D-ARABINOFURANOSYLCYTOSINE STIMULATES THYMIDINE INCORPORATION INTO THE DNA OF CONTACT-INHIBITED CELLS.

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Exp Cell Res; 135(2):427-435 1981

In rapidly proliferating cells 1-beta-D-arabinofuranosylcytosine (ara-C) is a potent inhibitor of DNA synthesis whose effect can be irreversible and consequently cytocidal. Whereas thymidine incorporation is greatly reduced in rapidly proliferating cells in the presence of ara-C, contact-inhibited cells, similarly treated, show increased thymidine incorporation by as much as seven-fold This ara-C induced stimula-

tion appears to result from an influence on thymidine utilization rather than increased DNA synthesis. (Author abstract) (20 Refs)

9. EFFECTS OF INHIBITORS OF DNA SYNTHESIS AND PROTEIN SYNTHESIS ON THE RATE OF DNA SYNTHESIS AFTER EXPOSURE OF MAMMALIAN CELLS TO ULTRAVIOLET LIGHT.

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Biochim Biophys Acta; 656(1):55-61 1981

Chinese hamster V-79 cells were treated with metabolic inhibitors of DNA or protein synthesis for various intervals of time after exposure of 3.0 or 5.0 J.m-2. After removal of the metabolic block(s) the rate of DNA synthesis was followed by measuring the incorporation of [14C]thymidine into acid-insoluble material. A 2.5 or 5.0 hr incubation with cycloheximide or hydroxyurea was effective in delaying the onset of the recovery in the rate of DNA synthesis that normally becomes evident several hr after exposur e to UV light. By using concentrations of cycloheximide or hydroxyurea that inhibit DNA synthesis by a similar amount (70%), but protein synthesis by vastly different amounts (95% for cycloheximide; 0% for hydroxyurea), it was apparent that the delay in recovery caused by the treatment of the cells with cycloheximide could be accounted for entirely by its inhibitory effect on DNA synthesis. This suggests that the recovery in DNA synthetic rates following exposure of V-79 cells to UV light does not appear to require de novo protein synthesis, and therefore does not appear to require the involvement of an inducible DNA repair process. (Author abstract) (27 Refs)

10. EFFECTS OF THE MYCOTOXIN BOTRYODIPLODIN ON MAMMALIAN CELLS IN CULTURE.

Moule Y, Douce C, Moreau S, Darracq N
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Chem Biol Interact; 37(1):155-164 1981

The effects of botryodiplodin, a mycotoxin synthesized by Botryodiplodia theobromae and by some strains of Penicillium roqueforti were studied. The toxin inhibited cell multiplication in growing cultures at concentrations which were without toxic effect on cultures nearing or at confluence. In growing cultures the toxin affected DNA, RNA and protein synthesis, the effect on DNA synthesis being the greatest. The removal of mycotoxin from the culture medium brought about partial recovery of the synthetic activities of the cells. The implications of the results with respect of the recovery of DNA synthesis are discussed. (Author abstract) (13 Refs)

11. 4,4'-DIMETHYLANGELICIN, A MONOFUNCTIONAL FUROCOUMARIN SHOWING HIGH PHOTOSENSITIZING ACTIVITY.

Baccichetti F, Bordin F, Carlassare F, Peron M, Guiotto A, Rodighiero P, Dall'Acqua F, Tamaro M
Inst. Pharmaceutical Chemistry, Padua Univ., Padua, Italy
Photochem Photobiol; 34(5):649-651 1981

A new angelicin derivative, 4,4'-dimethylangelicin, showed high photosensitizing activity. The compound had a high lethal effect in Escherichia coli B48, was mutagenic in E coli WP2, was phototoxic on guinea pig skin, and inhibited DNA synthesis in Ehrlich ascites tumor cells. (15 Refs)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

12. INHIBITION OF PURIFIED HUMAN AND HERPES SIMPLEX VIRUS-INDUCED DNA POLYMERASES BY 9-(2-HYDROXYETHOXYMETHYL)GUANINE TRIPHOSPHATE. EFFECTS ON PRIMER-TEMPLATE FUNCTION.

Derse D, Cheng YC, Furman PA, StClair MH, Elion GB
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J Biol Chem; 256(22):11447-11451 1981

The inhibition of highly purified herpes simplex virus (HSV)-induced and host cell DNA polymerases by the triphosphate form of 9-(2-hydroxyethoxymethyl)guanine (acyclovir; acycloguanosin e) was examined. Acyclovir triphosphate (acyclo-GTP) competitively inhibited the incorporation of dGMP into DNA, catalyzed by HSV DNA polymerase; apparent Km and Ki values of dGTP and acyclo-GTP were 0.15 uM and 0.003 uM, respectively. HeLa DNA polymerase alpha was also competitively inhibited; Km and Ki values of dGTP and acyclo-GTP were 1.2 uM and 0.18 uM, respectively. In contrast, HeLa DNA polymerase beta was insensitive to the analogue. The 'limited' DNA synthesis observed when dGTP was omitted from HSV or alpha DNA polymerase reactions was inhibited by acyclo-GTP in a concentration-dependent manner. Prior incubation of activated DNA, acyclo-GTP, and DNA polymerase (alpha or HSV) resulted in a marked decreased in the utilization of the primer-template in subsequent DNA polymerase reactions. This decreased ability of preincubated primer-templates to support DNA synthesis was dependent on acyclo-GTP, enzyme concentration, and the time of prior incubation. Acyclo-GMP-terminated DNA was found to inhibit HSV DNA polymerase-catalyzed DNA synthesis. Kinetic experiments with variable concentrations of activated DNA and fixed concentrations of acyclo-GMP-terminated DNA polymerase revealed a noncompetitive inhibition of HSV-1 DNA polymerase. The apparent Km of 3'-hydroxyl termini was 1.1 x 10(-7) M, the Kii and Kis of acyclo-GMP termini in activated DNA were 8.8 x 10(-8) M and 2.1 x 10(-9) M, respectively. Finally, 14C-labeled acyclo-GMP residues incorporated into activated DNA by HSV-1 DNA polymerase could not be excised by the polymerase-associated 3',5'-exonuclease activity. (Author abstract) (15 Refs)

13. INHIBITION OF CELLULAR AND VIRUS-ASSOCIATED NUCLEOTIDE POLYMERASES BY, AND ANTI-HERPES SIMPLEX VIRUS ACTIVITY OF, STREPTOVARICIN DERIVATIVES.

Srivastava BI, DiCioccio RA, Chadha KC, Rinehart KL
Dept. Experimental Therapeutics, Roswell Park Memorial Inst., Buffalo, NY, 14263
Antimicrob Agents Chemother; 20(5):653-659 1981

Fourteen streptovaricin derivatives were tested for inhibition of cellular nucleotide polymerases (DNA polymerase alpha, DNA polymerase beta, and DNA polymerase gamma, terminal deoxynucleotidyltransferase [TdT], and RNA polymerase II), simian sarcoma virus deoxyribonucleic acid polymerase, and herpes simplex virus Type 1-induced deoxyribonucleic acid polymerase (HSV-DP). Three compounds (streptovadienal C, prestreptovarone, and streptoval Fc) preferentially inhibited TdT and HSV-DP over the other enzymes. These compounds

inhibited HSV-DP more potently than they inhibited TdT. Evidence indicated that the mode of inhibition of TdT and HSV-DP by streptovadienal C and prestreptovarone was by interaction with the enzymes and not with template-primer, initiator, substrates, or divalent cations required for enzyme activity. Furthermore, data suggested that these compounds bind with greater affinity to HSV-DP than to TdT. Streptovadienal C and prestreptovarone were examined for their effect on the replication of herpes simplex virus Type 1 in African green monkey kidney (CV1) cells. These compounds produced 2-and 3-log drops in virus titer, respectively, at concentrations not significantly affecting cell viability. This correlated with evidence indicating a greater binding affinity of these compounds for HSV-DP over cellular nucleotide polymerases. (Author abstract) (27 Refs)

14. IDENTIFICATION OF A POLYPEPTIDE COMPONENT OF MOUSE MYELOMA DNA POLYMERASE GAMMA.

Matsukage A, Tanabe K, Yamaguchi M, Taguchi YN, Nishizawa M, Takahashi T, Takahashi T
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Biochim Biophys Acta; 655(3):269-277 1981

Mouse myeloma DNA polymerase gamma was extensively purified to a final specific activity of 156,000 units (nmol dTMP incorporation per hr) per mg protein on (rA)n.(dT)12-18 as a template primer. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of protein fractions obtained by DNA-cellulose column chromatography revealed that the amount of a polypeptide of Mr = 47,000 changed proportionally with DNA polymerase gamma activity. A minor polypeptide of Mr = 140,000 also seemed to change with the enzyme activity, but other polypeptides did not. Analysis by 125I-labeled peptide mapping indicates that the Mr 47,000 polypeptide in the mouse myeloma DNA polymerase gamma preparation is structurally related to the Mr 47,000 polypeptide of chick embryo DNA polymerase gamma. An antibody against chick embryo DNA polymerase gamma cross-reacted with the mouse enzyme, indicating a structural relationship between avian and murine enzymes. Since the Mr 47,000 polypeptide accounts for 31.4% of total protein in the purified preparation, the specific activity is estimated to be about 490,000 units per mg of the Mr 47,000 polypeptide. The rate of poly(dT) elongation by the purified enzyme was 1,260 nucleotides per min. This value is in the same range as the turnover number (1,530 nucleotides per min per enzyme molecule) which is calculated from the 'expected' specific activity with respect to the Mr 47,000 polypeptide and the mol wt (Mr = 188,000 on the assumption of a tetrameric structure of the Mr 47,000 polypeptide). Results indicate that the Mr 47,000 polypeptide is a component of the mouse myeloma DNA polymerase gamma. (Author abstract) (19 Refs)

15. STIMULATION OF DNA POLYMERASE ALPHA BY A NUCLEAR DNA/PROTEIN COMPLEX.

Balabanova H, Fridlender BR, Anderer FA
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J Supramol Struct Cell Biochem; 16(1):1-13 1981

A complex containing nuclear DNA, DNA polymerase (DPase), and simian virus 40 (SV40) T-antigen was isolated from nuclei of STU-A/232B transformed murine fibroblasts. Removal of DPase by ul trasonication and chromatographic separation left a DNA/T-antigen complex which stimulated the activity of DPase alpha approx 10-fold. Gel electrophoresis disclosed protein components of the complex with mol wt of 120,000, 94,000, 76,000, 54,000, and 46,000. Separation of DPase activity from T-antigen-specific complement fixation activity resulted in a loss of DPase activity. DPase-stimulation activity (DSA) was adsorbed by protein A-Sepharose loaded with specific IgG from SV40 tumor-bearing mice or antisera against the 94,000 or 76,000 mol wt complex fractions. DSA was partially inhibited by the same antisera. DSA was abolished by pretreatment with either trypsin or deoxyribonuclease I, but was not affected by heating to 80 C for 10 min. (49 Refs)

16. LOCALIZATION OF DNA METHYLTRANSFERASE IN THE CHROMATIN OF FRIEND ERYTHROLEUKEMIA CELLS.

Creusot F, Christman J K
Dept. Biochemistry, Mount Sinai Medical Center, 1 East 100th St., New York, NY, 10029
Nucleic Acids Res; 9(20):5359-5381 1981

Chromatin fragments released from intact Friend erythroleukemia cell nuclei during limited incubation with micrococcal nuclease, DNase II or DNase I were analyzed to determine the distribution of DNA methyltransferase in chromatin. The enzyme was released in a free form when internucleosomal DNA was digested with micrococcal nuclease but was found associated with Mg++-precipitable polynucleosomes after DNase II digestion. Less than 25% of the enzyme was released from nuclei incubated with DNase I under conditions where transcriptionally active chromatin should have been completely digested. These results indicated that the bulk of DNA methyltransferase was bound to 'linker' DNA in condensed regions of chromatin. Preferential rebinding of free enzyme to linker DNA was also demonstrated in vitro. The possibility that chromatin proteins play a role in regulating access of DNA methyltransferase to specific sites in DNA is discussed in light of these findings. (Author abstract) (38 Refs)

MECHANISMS OF DNA REPAIR

17. CORRELATIONS OF DNA DAMAGE AND REPAIR WITH NUCLEAR AND CHROMOSOMAL DAMAGE IN HELA CELLS CAUSED BY METHYLNITROSAMIDES.

Collins AR, Ord MJ, Johnson RT
Cancer Res. Campaign Mammalian Cell DNA Repair Group, Dept. Zoology, Univ. Cambridge, Downing St., Cambridge, CB2 3EJ, England
Cancer Res; 41(12):5176-5187 1981

N-Methyl-N-nitrosourethan induces breaks or alkali-labile sites in cellular DNA, many if not all of which are repaired rapidly. Other DNA lesions are repaired by an excision process. Hydroxyurea and 1-beta-d-arabinofuranosylcytosine cause an accumulation of DNA breaks after N-methyl-N-nitrosourethan treatment, probably by inhibiting the DNA-synthetic (but not the nucleolytic) stage of excision repair. Chromosome damage (fragmentation or attenuation of interphase chromosomes and de condensation and radial rearrangement of metaphase chromosomes) is present soon after treatment with N-methyl-N-nitrosourethan and is not reversed during further incubation. It is apparently associated with the longer-lived DNA lesions, probably those which are removed by excision, and is enhanced by incubation with hydroxyurea and 1-beta-d-arabinofuranosylcytosine. N-Methyl-N-nitrosourethan also in-

hibits cellular protein and DNA synthesis and causes the dispersion of polysomes and the loss of nucleolar structure. N-Methyl-N-nitrosourea is less potent than N-methyl-N-nitrosourethan in causing DNA or chromosome damage and is less cytotoxic. (Author abstract) (72 Refs)

18. APHIDICOLIN DOES NOT INHIBIT DNA REPAIR SYNTHESIS IN ULTRAVIOLET-IRRADIATED HELA CELLS: A RADIOAUTOGRAPHIC STUDY.

Hardt N, Pedrali-Noy G, Focher F, Spadari S
Departement de Biologie Moleculaire, Universite Libre de Bruxelles, Brussels, Belgium
Biochem J; 199(2):453-455 1981

A radioautographic examination of nuclear DNA synthesis in unirradiated and UV-irradiated HeLa cells, in the presence and in the absence of aphidicolin showed that aphidicolin inhibits nuclear DNA replication and has no detectable effect on DNA repair synthesis. Although the results establish that in uv-irradiated HeLa cells most of the DNA repair synthesis is not due to DNA polymerase alpha, they do not preclude a significant role for this enzyme in DNA repair processes. (Author abstract) (31 Refs)

19. EFFECTS OF INHIBITORS OF DNA STRAND BREAK REPAIR ON HELA CELL RADIOSENSITIVITY (MEETING ABSTRACT).

Ward JF, Blakely WF, Joner EI
Division of Radiation Biology, School of Medicine, University of California, San Diego, La Jolla, CA, 92093
Conference on Chemical Modification: Radiation and Cytotoxic Drugs, September 17-20, 1981, Key Biscayne, Florida. Committee for Radiation Oncology Studies 139 pp., 1981.

In order to assess the relationship between radiation induced cell killing and DNA single strand break repair capability, we have examined the effects of drugs shown to inhibit the latter process on cell radiosensitivity. For determination of DNA strand break repair rates we have developed the Kohn alkaline elution assay so that we can measure repair after low radiation doses (1 Gray, Gy) reproducibly and accurately. Using this assay we have shown that there are at least two components of strand break repair in V79 cells. The fast component after 1.5 Gy has a half time of approx 3.2 min and the slow of approx 50 at 24C. The fast component repair process is slowed at higher doses of radiation. The protocol developed for drug exposure was designed to inhibit DNA repair for the first hr following radiation: Aliquots of log phase HeLa cells were cooled to 24C 1 hr prior to irradiation at which time the drug (Cytosine arabinoside, N-ethylmaleimide, dinitrophenol, or diamide) was added. Cells were irradiated at OC and then incubated at 24C for 1 hr post irradiation with drug. The drug was then removed and cells plated out at 37C for assay of survival in the normal way. DNA strand break repair was measured as a function of post irradiation time at 24C. We observed that all four drugs can inhibit the fast strand break repair process at concentrations which are not cytotoxic. For instance cytosine arabinoside slowed the rate of fast strand break repair by a factor of 3. Under drug conditions in which fast strand break repair is inhibited, cells are radiosensitized: 10(-4) M cytosine arabinoside reduces DO from 1.3 Gy to 0.75 Gy and Dq from 1.8 Gy to 1.3 Gy. Thus while the proposed molecular mechanisms of drug action (in causing inhibition of DNA synthesis) are different, their common effect in causing inhibition of DNA strand repair correlates to their ability to increase cell radiosensitivity. (2 Refs)

20. DISTRIBUTION OF DNA-BOUND CARCINOGEN 4-NITROQUINOLINE 1-OXIDE AND OF REPAIR-SYNTHESIZED DNA IN CHROMATIN OF WI-38 CELLS.

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Dept. Biochemistry, Toyama Medical and Pharmaceutical Univ. Sch. Medicine, Sugitani, Toyama 930-01, Japan
Cancer Lett; 14(2):205-215 1981

Distribution within chromatin of the binding sites of 4-nitroquinoline-1-oxide (NQ) was investigated. Binding of NQ to nucleic acids of cells treated in vitro (WI-38 human diploid fibroblasts) reached a plateau by 90 min at a concentration of 1 x 10(-5) M. The specific activity of (3H)NQ bound to DNA did not change significantly during digestion with DNAase I, whereas it decreased with increasing degradation of DNA by micrococcal nuclease. These results suggest that NQ preferentially binds to nucleosomal linker DNA; no correlation is apparent between transcriptional activity of chromatin and binding of NQ to DNA. When the distribution of repair-synthesized DNA in chromatin damaged by NQ was studied, (3H)thymidine incorporated into repaired DNA was found to be more sensitive to nucleases but distributed approx equally among chromatin subfractions of differing transcriptional activity. (35 Refs)

21. QUANTITATION OF THE REPAIR OF GAMMA-RADIATION-INDUCED DOUBLE-STRAND DNA BREAKS IN HUMAN FIBROBLASTS.

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Biochim Biophys Acta; 655(3):342-348 1981

The quantitation and repair of double-strand DNA breaks in human fibroblasts has been determined using a method involving the nondenaturing elution of DNA from a filter. DNA from cells from two human fibroblast lines exposed to gamma-radiation from 0 to 10,000 rad showed increasing retention on a filter with decreasing radiation dose, and the data suggest a linear relationship between double-strand breaks induced and radiation dose. The ability of normal human fibroblasts to repair double-strand breaks with various doses of radiation was demonstrated, with a t1/2 of 10 min for repair of 5,000 rad exposure and 39 min for repair of 10,000 rad damage. The kinetics of the DNA rejoining were not linear and suggest that, as in the repair of single-strand breaks, both an initial 'fast' and a later 'slow' mechanism may be involved. (Author abstract) (17 Refs)

22. EXCISION REPAIR OF AFLATOXIN B1-DNA ADDUCTS IN HUMAN FIBROBLASTS.

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Cancer Res; 41(12):5125-5129 1981

The processing of covalent aflatoxin B1 (AFB1)-DNA adducts was investigated in confluent normal fibroblasts (NF) and xeroderma pigmentosum skin fibroblasts of Complementation Group A (XPA) following treatment with rat liver microsome-activated A1 for 30 min. Following rapid DNA isolation at slightly acidic pH by a new filter technique, more than 90% of the adducts corresponded to 2,3-dihydro-2-(N7-guanyl)-3-hydroxy-AFB1 (AFB1-N7-Gua) according to the analysis of acid hydrolysates by high-pressure liquid chromatography. The changes in adduct concentration and composition were compared between DNA isolated immediately following AFB1

treatment and incubated at neutrality in vitro and DNA in situ in the cell isolated after different lengths of incubation. The following conclusions were reached from the observed differences in the kinetics of adduct removal from free DNA and DNA in situ in NF and XPA: (a) AFB1-N7-Gua is removed spontaneously and enzymatically in NF but probably only spontaneously in XPA. This result suggests that these lesions are removed via nucleotide excision repair in NF; (b) the putative 2,3-dihydro-2-(N5-formyl-2',5',6'-triamino-4'-oxo-N5 pyrimidyl)-3-hydroxyaflatoxin B1 from AFB1-N7-Gua in vitro and in situ in the cell. It accumulates more rapidly and to a greater extent in XPA than in NF and persists in both cell types over prolonged periods. The reaction of A1-N2-Gua to 2,3-dihydro-2-(N5-formyl-2',5',6'-triamino-4'-oxo-N5 pyrimidyl)-3-hydroxyaflatoxin B1 represents the transformation of a repairable lesion to a non-repairable, persistent lesion. (Author abstract) (25 Refs)

23. REPAIR OF PSORALEN-TREATED DNA BY GENETIC RECOMBINATION IN HUMAN CELLS INFECTED WITH HERPES SIMPLEX VIRUS.

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Cancer Res; 41(12):5033-5038 1981

Herpes simplex virus type 1 was treated with 4,5',8-trimethylpsoralen (psoralen) plus near-UV light in order to produce lesions (monoadducts and DNA cross-links) in the viral DNA. Human fibroblasts were infected by damaged virus under conditions in which either a single virus particle or severa l particles entered a given cell, and the fraction of virus-producing cells was determined. This fraction was significantly greater for multiply infected cells than for singly infected cells, indicating that the psoralen lesions are repaired more efficiently in the presence of homologous, damaged DNA (multiplicity reactivation). Evidence is presented that herpes simplex virus may code for functions which participate in its own repair, both during multiplicity reactivation and during repair which occurs in singly infected cells: (a) host cells deficient in repair of lesions induced by psoralen (xeroderma pigmentosum) or the DNA cross-linking agent mitomycin C (Fanconi's anemia) exhibited normal levels of multiplicity reactivation of psoralen-treated herpes virus; (b) while xeroderma pigmentosum cells have been previously shown to be deficient in repair of psoralen-treated adenovirus under conditions of single infection, herpes virus is repaired at near normal levels in these same cells. Recombination levels between genetically marked pairs of herpes viruses were found to increase after treatment of the parental viruses with psoralen, suggesting that psoralen damage stimulates genetic recombination. This stimulation provides convincing evidence for a repair pathway in which genetic recombination between damaged viral genomes can lead to the production of viable virus. (Author abstract) (28 Refs)

24. DETECTION OF DNA SINGLE-STRAND BREAKS PRODUCED DURING THE REPAIR OF DAMAGE BY DNA-PROTEIN CROSS-LINKING AGENTS.

Fornace AJ
Lab. Pathology, NCI, NIH, Bethesda, MD, 20205
Cancer Res; 42(1):145-149 1982

In this investigation, the combination of 1-beta-D-arabinofuranosylcytosine and hydroxyurea was used to inhibit the polymerase step of DNA excision repair. The DNA single-strand breaks (SSB), which accumulated in the presence of these agents, were measured by alkaline elution. With this approach, DNA SSB were detected in normal human fibroblasts after exposure to trans-platinum(II)diamminedichloride, formaldehyde or potassium chromate. These agents all share the common feature in that they induce DNA-protein cross-links in mammalian cells. In the case of trans-platinum(II)diamminedichloride or formaldehyde, the frequency of these SSB was markedly less in excision-deficient xeroderma pigmentosum cells. With chromate, a high level of SSB was induced in both normal and xeroderma pigmentosum cells; these results indicate that chromate damage to DNA is repaired by a mechanism different than the classical excision pathway since xeroderma pigmentosum cells responded normally. Several other agents were investigated with this approach, and no SSB were detected with nickel sulfate, 12-O-tetradecanoylphorbol-13-acetate or asbestos fibers in the presence or absence of the polymerase inhibitor. This approach was found to be a very sensitive method to d etect DNA excision repair. (Author abstract) (21 Refs)

25. THE CHEMICAL INHIBITON OF PLD (POTENTIALLY LETHAL DAMAGE) REPAIR IN VITRO AND IN VIVO IN CANCER THERAPY (MEETING ABSTRACT).

Raymond U, Nakatsugawa S, Kumar A, Takahashi M, Ono K, Abe M, Nagata H, Sugahara T
Department of Radiology, Duke University Medical Center and Veterans Administration Medical Center, Durham, NC, 27710
Conference on Chemical Modification: Radiation and Cytotoxic Drugs, September 17-20, 1981, Key Biscayne, Florida. Committee for Radiation Oncology Studies 139 pp., 1981.

As a part of the cooperative research in cancer therapy, we are investigating the role of PLD repair in radioresistant tumor cells and inhibition of such repair by chemical means. The survival ratios of Chinese hamster HA-1 cells irradiated in a stationary phase and per cent of recovery inhibition as an index of efficiency on inhibition of PLD repair with Cordycepin (3'-deoxyadenosine) and its derivative analogues were investigated. Our results show that there was a weak but appreciable inhibitory effect on PLD repair by misonidazole treatments following radiation, whereas caffeine had a more potent inhibitory effect. However, postirradiation treatments with Cordycepin, or some of its derivatives, have shown a most significant effect in stationary phase of cells; which are known to exhibit radioresistance and greatest PLD repair capacity. Encouraged with the above results, id transplanted EMT-6 tumors in the right thighs of 8 wk old BALB/C female mice were used. When the tumor reached 5.5-7.5 mm diameter in size, they were then locally irradiated with 32 MeV electron beams. TCD 50/120 assay was used to evaluate the efficiency of 3'-deoxyadenosine and its derivatives given 100 mg/kg injection via ip route immediately after irradiation or following treatment with anti-tumor drugs such as Bleomycin. The 3'-deoxyadenosine (CDC) enhancement ratio, or DMF (dose-modifying factor) was 1.5, 3'-deoxyguanosine DMF was 1.9, and DMF for the CDC derivative identified as 1704 was 2.2 respectively. Thus, the above experimental data with EMT-6 tumors in mice also shows a very significant inhibitory effect against repair of anti-tumor agent-induced PLD. (no Refs)

26. REPAIR OF O(6)-ETHYLGUANINE IN DNA BY A CHROMATIN FRACTION FROM RAT LIVER: TRANSFER OF THE ETHYL GROUP TO AN ACCEPTOR PROTEIN.

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Proc Natl Acad Sci USA; 78(11,part2):6766-6770 1981

Incubation of O6-[3H]ethylguanine-containing DNA with a rat liver chromatin fraction resulted in a decrease in the O6-ethylguanine content of the DNA. Analysis of the products of this reaction showed that the ethyl group had been transferred from the O6-ethylguanine to a protein acceptor. When the incubation mixture was separated on a cesium chloride gradient, the radioactivity removed from O6-ethylguanine appeared in a low-density band. This material has been isolated and subjected to trypsin digestion and high-pressure liquid chromatography analysis; it was sensitive to trypsin and the digest contained new high-pressure liquid chromatography peaks characteristic of oligopeptides. Radioactive peaks from the trypsin digestion have been digested further to the amino acid level and have been shown to contain S-[3H]ethylcysteine. Thus, we conclude that the repair activity in rat liver chromatin removes the ethyl group from O6-ethylguanine and transfers it to a cysteine moiety contained in an acceptor protein. (Author abstract) (23 Refs)

27. EFFECTS OF 5-METHYLNICOTINAMIDE ON MOUSE L1210 CELLS EXPOSED TO N-METHYL-N-NITROSOUREA: MUTATION INDUCTION, FORMATION AND REMOVAL OF METHYLATION PRODUCTS IN DNA, AND UNSCHEDULED DNA SYNTHESIS.

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Carcinogenesis; 2(10):1013-1017 1981

Alkylation of L1210 DNA by N-methyl-N-nitrosourea (MNU) was measured, and the effects of 5'-methylnicotinamide (5MeN) on removal of methylated purines, on unscheduled DNA synthesis, and on induction of 6-thioguanine (6TG)-resistant mutants were examined. The loss of colony-forming ability of L1210 cells caused by MNU was potentiated 2.8-fold in the presence of 1 milliM 5MeN. 5MeN reduced the frequency of 6TG-resistant mutants induced by MNU: In two experiments, the induced mutation frequencies of cells not treated with 5MeN were 15.6 and 12.1 mutants per 10(4) surviving cells per milliM MNU compared with 7 mutants per 10(4) survivors per milliM MNU for mutations expressed and selected in the presence of 5MeN. 7-Methylguanine removal from DNA by MNU was unaffected by 5MeN, whereas O6-methylguanine (O6-MeG) was removed from 5MeN-treated cells at a significantly higher rate (half-life 28 hr) than from untreated cells (half-life 46 hr). 5MeN had no effect on inhibition of normal replicative DNA synthesis by 0.2 milliM hydroxyurea, whereas it stimulated unscheduled DNA synthesis under similar conditions. The observation that 5MeN reduces the frequency of 6TG-resistant mutants induced by MNU and enhances the rate of O6-MeG removal is consistent with this being a promutagenic lesion in L1210 cells. (48 Refs)

28. ABNORMAL RECOVERY OF DNA REPLICATION IN ULTRAVIOLET-IRRADIATED CELL CULTURES OF DROSOPHILA MELANOGASTER WHICH ARE DEFECTIVE IN DNA REPAIR.

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MGG; 183(2):363-368 1981

Cell cultures prepared from embryos of a control stock of Drosophila melanogaster respond to UV light with a decline and subsequent recovery both of thymidine incorporation and in the abili ty to synthesize nascent DNA in long segments. Recovery of one or both capacities is absent or diminished in irradiated cells from 10 nonallelic mutants that are defective in DNA repair and from four of five nonallelic mutagen-sensitive mutants that exhibit normal repair capabilities. Recovery of thymidine incorporation is not observed in 9 of 10 DNA repair-defective mutants. On the other hand, partial or complete recovery of incorporation is observed in all but one repair-proficient mutagen-sensitive mutant. Irradiated cells from two mutants that display no excision capacity exhibit a gradual arrest of thymidine incorporation within 20 hr after the initial decline. this arrest of incorporation is not observed in mutants exhibiting only partial defects in excision repair. Recovery of the ability to synthesize nascent DNA in long segments is normal in cells from the two mutants that display no excision capacity, indicating that recovery does not depend upon the excision of pyrimidine dimers from cellular DNA. Recovery of that ability is not observed, however, in cells from one partially excision-defective mutant, two of three postreplication repair-defective mutants, two of four mutants defective in both excision and postreplication repair, and one of five repair-proficient mutagen-sensitive mutants. These results indicate that recovery of normal DNA replication in irradiated Drosophila cells depends upon the activity of several functions. (Author abstract) (17 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

29. DNA METHYLATION IN NORMAL AND SV40-TRANSFORMED HUMAN FIBROBLASTS.

Diala ES, Plent MM, Coalson DW, Hoffman RM

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Biochem Biophys Res Commun; 102(4):1379-1384 1981

The results of high performance liquid chromatographic studies in four normal and four simian virus 40 (SV40)-transformed human diploid fibroblast cultures indicated that SV40 transformation does not affect the extent of total genomic DNA methylation in human fibroblasts. This is in contrast with the results of other studies, which suggest that transforming viruses do affect the extent of total genomic methylation. The possibility that SV40 transformation causes changes in site-specific DNA methylation in human cells remains to be investigated. (18 Refs)

30. DEOXYRIBONUCLEIC ACID METHYLATION IN HUMAN CELLS EXPOSED TO NITROSOCIMETIDINE.

Jensen DE
Fels Res. Inst., Temple Univ. Sch. Medicine, Philadelphia, PA, 19140
Biochem Pharmacol; 30(20):2864-2867 1981

DNA methylation rates and methylated purine yields are reported for Raji cells exposed to nitrosocimetidine (NC), methylnitrosourea, and N-methyl-N'-nitro-N-nitrosoguanidine. NC was unique relative to the other two compounds in that it hydrolyzed only very slowly in cellular growth medium and m ethylated DNA in rapidly growing cells slowly but persistently. (26 Refs)

31. VERTEBRATE DNAS CONTAIN NUCLEOTIDE SEQUENCES RELATED TO THE TRANSFORMING GENE OF AVIAN MYELOBLASTOSIS VIRUS.

Bergmann DG, Souza LM, Baluda MA
Univ. California Los Angeles Sch. Medicine, Los Angeles, CA, 90024
J Virol; 40(2):450-455 1981

Avian myeloblastosis virus contains a continuous sequence of approx 1,000 nucleotides which may represent a gene (amv) responsible for acute myeloblastic leukemia in chickens. This sequence appears to have been acquired from chicken DNA and to be substituted for the envelope gene in the viral genome. We used hybridization probes enriched for the amv sequences and conditions that facilitate annealing of partially homologous nucleotide sequences to show that cellular sequences related to amv are present in the genomes of all vertebrates ranging from amphibians to humans but were not detected in fish, sea urchins, or Escherichia coli. In contrast to the preceding findings, nontransforming endogenous proviral nucleotide sequences closely related to the remainder of the avian myeloblastosis virus genome and to the entire myeloblastosis-associated helper virus are present only in chicken DNA. The amv-related cellular sequences appear to be highly conserved during evolution and to be contained at only one or a few locations in the genome of vertebrates. Within closely related species, they appear to share common evolutionary genetic loci. These findings and similar ones obtained with other highly oncogenic retroviruses containing a transforming gene suggest a general mechanism for acquisition of viral oncogenic sequences and an essential role for these sequences in the normal cellular state. (Author abstract) (29 Refs)

32. COMPARISON BETWEEN THE VIRAL TRANSFORMING GENE (SRC) OF RECOVERED AVIAN SARCOMA VIRUS AND ITS CELLULAR HOMOLOG.

Takeya T, Hanafusa H, Junghans RP, Ju G, Skalka AM
Rockefeller Univ., New York, NY, 10021
Mol Cell Biol; 1(11):1024-1037 1981

To gain information on the relationship between the src region of Rous sarcoma virus (RSV) (v-RSV) and cellular src (c-src), and clues to the origins of recovered avian sarcoma viruses (rASV), molecular clones were isolated containing src related sequences from the parental RSV, from rASV, and from c-src, the presumed progenitor of rASV. Molecular clones of viral DNA from rASV and its transformation-competent progenitor, the Schmidt-Ruppin A strain of RSV were constructed and analyzed. A 2.0-megadalton EcoRI fragment, containing the entire src gene from each of the clones was subcloned and characterized. The fragments were also used as probes to isolate recombinant phage clones containing the cellular counterpart of the viral src gene (c-src), from a lambda library of chicken DNA. Restriction endonuclease mapping of c-src revealed extensive similarity between the src region of RSV and rAVS, but striking differences between viral src's and c-src. Electron microscopic analysis of heteroduplexes between recovered virus src and c-src revealed a 1.8-kilobase homologous region. In the cellular gene, the homologous region was interrupted by seven nonhomologous regions which is interpreted to be intervening sequences. Minimum length of c-src is estimated at 7.2 kilobases. The findings have implications concerning the mechanism of formation of recovered virus src and possibly other cell-derived retrovirus transforming genes. (58 Refs)

33. MAPPING OF IMMUNOGLOBULIN VARIABLE REGION GENES: RELATIONSHIP TO THE 'DELETION' MODEL OF IMMUNOGLOBULIN GENE REARRANGEMENT.

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Nucleic Acids Res; 9(21):5725-5735 1981

Five families of variable region genes of mouse kappa chains were analyzed by Southern blot hybridization to determine their relative chromosomal map positions. Map positions were deduced by Vkappa gene deletion from antibody-producing cells expressing upstream Vkappa genes and retention in cells expressing downstream genes. The Vkappa regions expressed in the myelomas MOPC167, MPC11, MOPC21 and ABPC20 are members of Vkappa families exhibiting one, three, six and six major germline hybridization bands respectively. The gene order of the five families in germline DNA was found to be VM167-VM11-(VM21, VA20) - VABE8-Jkappa - Ckappa. As expected in a deletion model of Ig gene rearrangement, a sequence located just 5' of J1 in germline DNA was found to be absent from some antibody producing cells which had not retained any germline Ckappa genes. However, other cell lines contained this sequence in rearranged contexts, suggesting that any deletion model of Ig V-J joining, as well as V gene mapping, must take into account the possibilities of stepwise rearrangements and reintegration of 'deleted' DNA. (Author abstract) (17 Refs)

34. RNA SPLICING MUTATION IN AN ABERRANTLY REARRANGED IMMUNOGLOBULIN LAMBDAI GENE.

Hozumi N, Wu GE, Murialdo H, Roberts L, Vetter D, Fife WL, Whiteley M, Sadowski P
Ontario Cancer Inst., Univ. Toronto, Toronto, Canada
Proc Natl Acad Sci USA; 78(11,part2):7019-7023 1981

The mouse cell line MOPC 315 is an IgA (lambdaII)-producing myeloma. We have studied a derivative of MOPC 315 that secretes normal lambdaII chains but no heavy chain. This derivative, MOPC 315-26, was found to contain a rearranged lambdaI gene in addition to a rearranged lambdaII gene. The rearranged lambdaI gene was cloned into bacteriophage lambda DNA and its structure was studied. The lambdaI gene was found to have arisen by an aberrant recombination event that resulted in a single base insertion at the sit e of V-J region joining. In addition, the gene contained numerous point mutation in the vicinity of the junction of the V and J regions. Two point mutations occurred in the donor splice sequence normally used for the removal of the intron between the J and C regions, suggesting that the RNA synthesized from the aberrantly rearranged lambdaI gene would be unable to undergo proper RNA splicing. (Author abstract) (38 Refs)

35. ENHANCING EFFECT OF MURINE LEUKEMIA VIRUS ON FIBROBLAST TRANSFORMATION BY NORMAL BALB/C DNA FRAGMENTS.

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Haematol Blood Transfus; 26:479-482 1981

Mechanically fragmented normal BALB/c mouse DNA was used to transform NIH/3T3 cells. Primary transformation occurred at a low frequency, but secondary transfections with DNA isolated from the transformed cells was highly efficient. Appropriate controls were used to ensure the DNA specificity of this phenomenon. No reverse transcriptase activity was found in any of the transformed cell lines. All six transformed cell lines were anchorage independent (they grew well in soft agar) and all were oncogenic when injected ip into newborn athymic BALB/nude mice. Five of the six cell lines produced fibrosarcomas within 3 wk when injected sc into 3-wk-old BALB/c mice. No transforming virus could be rescued after infection of the cell lines with Moloney strain murine leukemia virus (MuLV). Antibody raised in C57BL mice against syngeneic Abelson MuLV (A-=MuLV) reacted with a variety of A-MuLV-transformed cells but not with any of the NIH/3T3 cells infected with Moloney MuLV. Preinfection of the NIH/3T3 cells with either Rauscher or Moloney MuLV enhanced transformation by normal cell DNA fragments (transformation occurred both earlier and with a higher efficiency). These and previously reported finding support the idea that the insertion of cellular genes in transposed sequence into the cellular DNA may result in oncogenic transformation, perhaps due to their being inserted near active promoter sequences. It is further hypothesized that preinfection with MuLV produces a semitransformed state in 3T3 cells or that MuLV provides the infected cells with highly active promoter sequences. (7 Refs)

36. THE MECHANISM OF ACTION OF PLATINUM-CONTAINING ANTITUMOR DRUGS: DNA INTERACTIONS.

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Diss Abstr Int [B]; 42(3):911-B 1981

The mechanism of action of the potent antitumor drug cis-dichlorodiammineplatinum(II) (cis-DDP) has been examined. Maxam-Gilbert Nucleotide sequencing and DNase footprinting demonstrate that cis-DDP binds to sequential guanine sequences on one strand of a DNA restriction enzyme fragment from the origin of replication of a bacterial plasmid. The clinically inactive trans isomer exhibit s no sequence specificity. This is consistent with the proposed mechanism of action whereby cis-DDP causes intrastrand crosslinking of adjacent guanine residues. Because of its geometry, the trans isomer cannot participate in an analogous intrastrand crosslinking reaction. Agarose gel electrophoresis of platinum-DNA complexes demonstrates that cis-DDP binds to DNA by two distinct modes, whereas trans-DDP binds by only one mode. These experiments establish, in addition, that cis-DDP promotes unwinding of the duplex. Digestion with the single-strand specific endonuclease S1 reveals that single-stranded regions are concomitantly generated. SV40 large Tumor antigen (TAg) is required to initiate SV40 DNA replication; this protein binds specifically to the unique origin of replication of SV40 DNA. DNase protection experiments demonstrate that cis-DDP inhibits the specific binding of SV40 TAg to the origin of replication of SV40 DNA. These studies indicate that although both cis- and trans-DDP bind to DNA, only cis-DDP binds by a mechanism consistent with intrastrand crosslinking of adjacent guanine bases. This cis-DDP-specific reaction rapidly changes the physical characteristics of the DNA. These perturbations in DNA structure inhibit the interaction between a virus-coded protein required for DNA replication and the modified viral DNA template. (no Refs)

37. BASE-PAIR SUBSTITUTION HOTSPOTS IN GAG AND GCG NUCLEOTIDE SEQUENCES IN ESCHERICHIA COLI K-12 INDUCED BY CIS-DIAMMINEDICHLOROPLATINUM (II).

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Proc Natl Acad Sci USA; 78(11,part2):7010-7014 1981

Cell killing and mutation induction by cis- and trans-PT(NH3)2CL2 in Escherichia coli were examined by studying forward mutagenesis in the lacI gene in cells with different repair capacities. Survival experiments showed that repair-proficient cells were slightly more sensitive for the cis isomer than for the trans isomer, whereas repair-deficient RecA and UvrB cells were extremely sensitive only for the cis compound. cis-Pt(NH3)2Cl2 induced mutagenesis in both wild-type cells and RecA cells but not in UvrB cells; whereas no detectable mutagenesis was induced by treatment with the trans compound. Examination of the nature of the mutations induced by cis-PT(NH3)2Cl2, by using the LacI system, revealed that base-pair substitutions leading to nonsense mutants are only induced in wild-type cells, suggesting that the intact products of both the uvrB and the recA gene are necessary for the repair responsible for this type of mutagenesis. Investigation of the nonsense mutants reveals that 70% of these mutations result from GC - TA or GC - AT substitutions at sites where the guanine is part of a GAG or GCG sequence. These results are discussed in relation to existing theories on the interaction between Pt compounds and DNA. A model for Pt-DNA adducts, leading to base-pair substitutions, is proposed. (Author abstract) (50 Refs)

38. BASE INSERTION AND DELETION MUTATIONS INDUCED IN AN ESCHERICHIA COLI PLASMID BY BENZO[A]PYRENE-7,8-DIHYDRODIOL-9,10-EPOXIDE.

Mizusawa H, Lee CH, Kakefuda T, McKenney K, Shimatake H, Rosenberg M
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Proc Natl Acad Sci USA; 78(11,part2):6817-6820 1981

Mutations induced by (+-)trans-benzo-[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BaP-diol epoxide-1) were selected by using a recombinant plasmid vector system designed for the study of transcription termination signals. The plasmid contains a transcription terminator positioned between a promotor signal and the Escherichia coli galactokinase structural gene (galK). By selections for the expression of galK (ie, galK- to galK+), mutations are obtained in the terminator region that allow transcription from the promotor to read the galK gene. These mutations were characterized by direct DNA sequence of the terminator region. The DNA sequence changes caused by BaP-diol expoxide-1 were demonstrated for three different mutants. Two were found to be single-base-pair insertions of T-A into a cluster of consecutive T-A base pairs and the other change was a single-base-pair deletion of G-C from a cluster of consecutive G-C base pairs. (Author abstract) (30 Refs)

OTHER RELATED STUDIES

39. IMMUNOCYTOLOGICAL DETECTION OF AAF-DNA ADDUCTS IN HELA CELL NUCLEI.

Sage E, Gabelman N, Mendez F, Bases R
(c/o Bases) Dept. Radiology, Albert Einstein Coll. Medicine, Bronx, NY, 10461
Cancer Lett; 14(2):193-204 1981

Rabbit antibodies for acetylaminofluorene (AAF)-guanosine and for DNA modified by the presence of AAF adducts were utilized. HeLa cells growing in suspension at 2.5 x 10(5)/ml were exposed to increasing concentrations of N-acetoxy-AAF in dimethylsulfoxide at less than 0.5% final concentration. Dose-dependent increase in the proportion of immunoreactive cells in cultures exposed to carcinogen for 15 min at 37 C was observed. When the time course of disappearance of nuclear immunoreactivity toward anti-AAF-DNA was compared with the loss of radiolabel in cells exposed to 5 ug/ml of labeled N-acetoxy-AAF, nuclear immunoreactivity was induced but then declined rapidly for 5 hr. The total radiolabel in purified nuclear DNA declined much more slowly. In the first 3 hr after removal of carcinogen, the induced immunoreactivity was rapidly lost with no significant reduction in labeled DNA or in the dominant adduct fraction, N-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AAF). In the next 3 hr loss of immunoreactivity was insignificant but the drop in acetylated C-8 adducts was dramatic. Radiolabel experiments indicated that most adducts were st ill within the nucleus but did not react with antibody. It is suggested that DNA-AAF adducts become inaccessible to antibody due to the mechanics of the repair process. (33 Refs)

40. SIMULTANEOUS EXAMINATION OF SISTER CHROMATID EXCHANGES AND CELL REPLICATION KINETICS IN TUMOR AND NORMAL CELLS IN VIVO.

Schneider EL, Nakanishi Y, Lewis J, Sternberg H
Section Cellular Aging and Genetics, Lab. Cellular and Molecular Biology, Gerontology Res. Center, Natl. Inst. Aging, NIH, Baltimore, MD, 21224
Cancer Res; 41(12):4973-4975 1981

The bromodeoxyuridine differential chromatid labeling techniques were applied to the simultaneous in vivo examination of cell cycle kinetics and sister chromatid exchange (SCE) induction by various cancer chemotherapeutic agents in normal and Ehrlich ascites tumor cell populations. Compounds which yielded high ratios of SCE (in tumor cells):SCE (in normal cells) also produced greater inhibition of cellular replication in tumor cells when compared to normal cells. Thus, this approach permits in vivo assessment of induction of DNA damage by SCE analysis as well as inhibition of cellular replication by specific cancer chemotherapeutic agents in tumor and normal cell populations. (Author abstract) (21 Refs)

41. STIMULATION OF ADP-RIBOSYLATION OF NUCLEAR PROTEINS IN PERMEABILIZED CELLS AFTER TREATMENT OF MOUSE LYMPHOMA (L1210) CELLS WITH THE ALKYLATING AGENT, DIMETHYL SULPHATE (MEETING ABSTRACT).

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Cell Biol Int Rep; 5(SupplA):38 1981

ADP-ribosylation of nuclear proteins has been studied in a number of cell types in both isolated nuclei and to a much lesser extent in intact cells. One physiological function of (ADP-ribose) seems to be in DNA repair; there have been suggestions of an involvement in cell differentiation. Intact cells are impermeable to NAD. 3H-NAD incorporation into L1210 cells permeabilized by hypotonic treatment was less than 4% of that into isolated nuclei, but the former is, we suggest, more representative of the situation in intact cells. For example, polyADPR-glycohydrolase (which degrades polyADPR into monoADPR) in permeabilized cells was almost as high as in intact cells, but very much reduced in nuclei. After damaging the cellular DNA by dimethyl sulphate (DMS) treatment, a 9-fold increase in 3H-NAD incorporation into acid-insoluble material after permeabilization was observed relative to controls not treated with the alkylating agent. Histones represented 7-10% of the total acid-insoluble radioactivity, which was increased 20-fold by DMS. A further 70% was extractable by 9M urea-2% NP40 and this was increased 7-fold by DMS. The radioactive proteins have been analyzed on polyacrylamide gels. Treatment of the acid-insoluble radioactiv ity for 15 min with either snake venom phosphodiesterase (PDE) or glycohydrolase rendered 83-88% acid-soluble, whereas a DNAse/RNAse mixture had no effect (although it did render 88% of 3H-uridine-labelled nucleic acids acid-soluble under the same conditions). Complete digestion with PDE produced 5'-AMP and phosphoribosyl-AMP (unique to polyADPR) on PEI thin-layer chromatographs and enabled determination of av polyADPR chain length. The results support a role for polyADP - ribosylation in DNA repair. (no Refs)

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SERIES CB25 No. 82/05

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EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

1. SUPPRESSOR CELL ACTIVITY IN TUMOR-BEARING MICE. III. CO-PURIFICATION OF A FACTOR INHIBITING CELLULAR DNA SYNTHESIS AND DNA POLYMERASE ACTIVITY.

Farrar WL, Elgert KD, Foo AS
(c/o Elgert) Dept. Biology, Microbiology Section, Virginia Polytechnic Inst. and State Univ., Blacksburg, VA, 24061
J Immunol; 127(6):2339-2344 1981

The factor(s) derived from fibrosarcoma-induced suppressor T cells was sensitive to pronase and neuraminidase, but not to trypsin, beta-galactosidase, DNase, or RNase. Protein and RNA, but not DNA, synthesis were required to mediate suppression. Suppressor T cell-derived factor(s) could be precipitated by a 50% saturated ammonium sulfate (SAS) solution. The 50% SAS fraction inhibited both in vitro and in vivo spleen cell blastogenesis, whereas the 80% and unprecipitated fractions had no inhibitory activity. Using Sephadex G-200 chromatography, the 2nd protein fraction (Fraction II) contained an inhibitor of both DNA polymerase (IDP) and DNA

HE 20.3173/2:

synthesis (IDS) activity, which possessed no cytotoxic activity. In vitro DNA polymerase alpha activity was suppressed by Fraction II, whereas DNA polymerase beta and gamma activities remained unchanged. Mol wt of IDP/IDS, as determined by Sephadex G-200 gel filtration chromatography, was approx 14,500. Attempts to separate IDP/IDS activities found in Fraction II by anion-exchange chromatography and slab gel electrophoresis were not successful, which suggested that the two activities were the same or very similar molecules. (Author abstract) (26 Refs)

2. STIMULATION OF NA INFLUX, NA-K PUMP ACTIVITY AND DNA SYNTHESIS IN QUIESCENT CULTURED CELLS.

Rozengurt E
Imperial Cancer Res. Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, England
Adv Enzyme Regul; 19:61-85 1981

A central problem in cell regulation is to elucidate the biochemical basis of the metabolic activation seen in quiescent cells stimulated to proliferate. Recent evidence has shown that an increase in the rate of Na influx is one of the earliest events in the stimulation of quiescent cells into DNA synthesis and cell division. This phenomenon has been demonstrated in a variety of fibroblastic cells and it is elicited by serum, as well as by purified growth factors. The stimulation of Na influx is apparently sufficient to increase the activity of the Na-K pump of the plasma membrane. The activity of this enzyme is sensitive to small fluctuations of cellular Na, as shown by using the selective Na ionophore monensin, which increases cellular Na and Na-K pump activity. A number of recent findings suggest that the influx of Na ions into the cell can signal later events of cell proliferation, specifically the initiation of DNA synthesis. The evidence supporting this notion is as follows: (a) the rate of Na entry rapidly increases in a variety of quiescent fibroblastic cells stimulated to proliferate by serum or purified growth factors; (b) a decrease in Na entry by lowering the Na concentration in the extracellular medium or by inhibiting the uptake of Na by amiloride markedly blocks the initiation of DNA synthesis promoted by serum or growth factors; (c) the neurohypophyseal nonpeptide vasopressin, the potent tumor promoter TPA, the amphipathic polypeptide melitin and the channel-forming antibiotic amphotericin B stimulate Na ion influx and trigger the initiation of DNA synthesis, acting synergistically with other growth factors; (d) certain cells transformed by oncogenic viruses show an increased Na flux as compared with their normal counterparts. These findings suggest that Na influx may signal the initiation of mitogenesis in quiescent cells. (Author abstract) (84 Refs)

3. HORMONE REGULATION OF DNA SYNTHESIS OF NORMAL AND HYPERPLASTIC HUMAN BREAST TISSUES IN VITRO AND IN VIVO (MEETING ABSTRACT).

Welsch CW
Department of Anatomy, Michigan State University, East Lansing, MI, 48824
International Symposium on Endocrinology of Cystic Breast Disease, November 5-6, 1981, Turin, Italy Italian Society of Endocrinology; :A3 1981

The hormones analyzed in these studies were bovine insulin (5.0 ug/ml), ovine prolactin (5.0 ug/ml), human prolactin (5.0 ug/ml), human placental lactogen (5.0 ug/ml), human growth hormone (5.0 ug/ml), 17beta-estradiol (0.001 ug/ml) and progesterone (1.0 ug/ml). Epithelial (ductal) DNA synthesis of the cultured human breast tissues was significantly stimulated by insulin, human prolactin, human placental lactogen and 17beta-estradiol; ovine prolactin, human growth hormone and progesterone were without a stimulatory effect. The original morphology of the explants was maintained. In vivo studies (athymic nude mice): Human breast tissue was obtained and processed as described above and transplanted sc (dorsal) to 8 wk old female athymic nude mice (Balb/c). Thirty days after transplantation, the mice were treated with the following hormones: human placental lactogen (0.5 mg; 2 x daily); estrogen (17beta-estradiol, 1.0 ug, 1 x daily and estrone in drinking water, 0.5 mg/liter) or a sc grafted rat pituitary tumor (RPT) which secretes copious quantities of prolactin and growth hormone (host mice blood rat prolactin levels, greater than 4000 ng/ml). After 30 days of hormone treatment, the grafted human breast tissues were excised and incubated for 4 hr in a 3H-thymidine enriched culture media. The grafts were processed for 3H-thymidine autoradiography. Human placental lactogen, estrogen and estrogen + RPT significantly stimulated DNA synthesis of the ductal epithelium of the grafted human breast tissues; RPT alone was ineffective. One hundred percent of the grafts were accepted by control or hormone treated mice; no sign of immunological rejection was observed. The grafts acquired the host blood supply and retained the original morphology. The relative merits of our in vitro and in vivo systems for the study of human breast tissues will be discussed. (no Refs)

4. AN ANTIFOLATE-INDUCED LESION IN NEWLY SYNTHESIZED DNA.

Sedwick WD, Laszlo J
Dept. Medicine, Duke Medical Center, Durham, NC, 27710
Adv Enzyme Regul; 19:295-308 1981

Antifolates cause cells to accumulate exogenous UdR as dUMP in drug-treated cells. This accumulation of intracellular dUMP has no effect on inhibition of UdR incorporation into DNA. On the other hand, dUMP accumulation does result in detectable dUTP being synthesized and utilized as substrate for DNA synthesis. Although in the presence of metoprine (DDMP), dUMP is incorporated into DNA instead of TMP, the natural product of UdR substrate in the absence of the drug, DNA synthesis is equally rate limited by exogenous UdR. However, when analyzed on alkaline sucrose gradients, DNA which has incorporated exogenous UdR in the presence of antifolate sediments at 4S or less. This newly synthesized DNA accumulates preferentially while either synthesis of larger DNA is blocked, or high mol wt DNA is preferentially broken down as a result of DNA repair. These observations further increase the metabolic complexity of pathways that must be considered as underlying the differential toxicity of antifolates. (Author abstract) (32 Refs)

5. DIFFERENTIAL SUSCEPTIBILITY OF CANCER AND NORMAL DNA TEMPLATES ALLOWS THE DETECTION OF CARCINOGENS AND ANTI-CANCER DRUGS (ONCOTEST) (MEETING ABSTRACT).

Beljanski M, Le Goff L, Beljanski MS
Laboratoire de Pharmacodynamie, 92290 Chatenay-Malabry, France
Third NCI-EORTC Symposium on New Drugs in Cancer Therapy, October 15-17, 1981, Brussels, Belgium European Organization for Research on Treatment of Cancer; :A91 1981

Based on the differential template activity exhibited by DNA isolated from cancerous and healthy human or animal tissues, the Oncotest is an accurate and rapid assay for the screening of

carcinogenic compounds. Thus, in the presence of all necessary components for radioactive DNA synthesis by DNA polymerase, carcinogenic compounds, antimitotic drugs (and miscellaneous compounds), at given concentrations strongly stimulate the synthesis of DNA isolated from different cancer tissues, but only slightly enhance that DNA from healthy tissues. Those carcinogens which cannot be characterized as mutagens in the Salmonella assay system, behave as carcinogens in the Oncotest. Carcinogenic potential of steroids can be shown by using the DNA from steroid hormone target tissue. There exists a common molecular mechanism through which carcinogens stimulate cancer DNA in vitro synthesis. Cancer DNAs are destabilized and in the presence of carcinogenic substances there is further DNA strand separation which accounts for the enhanced DNA synthesis. A correlation between in vitro cancer DNA synthesis, DNA strand separation and in vivo multiplication of cancerous cells can be demonstrated. Our assay system allows detection of those substances which selectively inhibit cancer DNA synthesis without affecting, in an appreciable way, that of DNA from healthy tissues. (no Refs)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

6. BIOCHEMICAL AND IMMUNOLOGICAL CHARACTERIZATION OF CELLULAR DNA POLYMERASES ALPHA, BETA AND GAMMA, AND A REVERSE TRANSCRIPTASE FROM HUMAN MELANOMA TISSUE.

Chandra P, Balikcioglu S, Mildner B
Zentrum der Biologischen Chemie, Theodor-Stern-Kai 7, D-6 Frankfurt a. Main 70, W. Germany
Cell Mol Biol; 27(2/3):239-251 1981

The present study describes separation and purification of a reverse transcriptase and cellular DNA polymerases from human primary melanoma tissue. The specific requirements with respect to bivalent cations and template-primers for DNA polymerase alpha, DNA polymerase beta and DNA polymerase gamma, as well as for the reverse transcriptase, are reported. Sodium dodecylsulfate gel electrophoresis of the purified enzymes gave estimated values of mol wt of 160,000, 38,000, 100,000 and 68,000 daltons for DNA polymerase alpha, DNA polymerase beta, DNA polymerase gamma and reverse transcriptase respectively. Serological studies have shown that the reverse transcriptase of human melanoma tissue is not antigenically related to cellular DNA polymerase alpha, beta, or gamma, but is antigenically related to reverse transcriptases from baboon endogenous virus (BEV) and RD-114. This is the first human reverse transcriptase showing cross-reactivity to DNA polymerases from endogenous retroviruses. (Author abstract) (24 Refs)

7. ASSOCIATION OF DIADENOSINE 5',5'-P,(1),P(4)-TETRAPHOSPHATE BINDING PROTEIN WITH HELA CELL DNA POLYMERASE ALPHA.

Rapaport E, Zamecnik PC, Baril EF
Dept. Microbiology, Boston Univ. Sch. Medicine, Boston, MA, 02118
J Biol Chem; 256(23):12148-12151 1981

An electrophoretically homogeneous high mol wt form (640,000) of HeLa cell DNA polymerase alpha was shown to catalyze DNA synthesis with a variety of di- and oligoriboadenylates and oligodeoxyriboadenylates as primers with poly(dT) as template. Diadenosine 5',5'-P1,P(4)-tetraphosphate (Ap4A) can be utilized as a primer with poly(dT) as template and was found to be covalently attached to the 5'-end of the poly(dA) product. An Ap4A binding protein is tightly associated with the high mol wt form of DNA polymerase alpha. This protein which exhibits high affinity, noncovalent binding of Ap4A is resolved from the multiprotein DNA polymerase alpha complex, along with other accessory proteins, by hydrophobic affinity chromatography on phenyl-Sepharose columns. (Author abstract) (25 Refs)

8. DNA POLYMERASE ACTIVITY IN BLOOD LYMPHOCYTES IN CATTLE WITH CHRONIC LYMPHOID LEUKEMIA.

Kovalevskii NA, Azizov IuM, Aleshchenko AV, Belov AD
Inst. Chemical Physics, Moscow, USSR
Izv Akad Nauk SSSR [Biol]; (6):899-909 1981

Activity of DNA polymerase was studied in peripheral blood lymphocytes from normal and chronic lymphoid leukemia (CLL) cattle. In the absence of the exogenous DNA, DNA polymerase activity in CLL lymphocytes was similar to that in controls. Incubation of lymphocytes with 3H-thymidine triphosphate resulted in marked increase of enzyme activity in CLL lymphocytes. Radioautographic and cytofluorimetric determinations of the labeling index and DNA content showed that the rate of DNA proliferation in CLL lymphocytes was comparable to that in controls. These findings indicated that the increase of DNA polymerase a ctivity in CLL lymphocytes was associated not with DNA proliferation per se but rather with the increase of the content of alpha-DNA polymerase. (17 Refs) (In RUS)

9. DETECTION OF TERMINAL TRANSFERASE IN PARAFFIN SECTIONS WITH THE IMMUNOPEROXIDASE TECHNIQUE.

Halverson CA, Falini B, Taylor CR, Parker JW
Dept. Pathology, Univ. Southern California Sch. Medicine, Los Angeles, CA
Am J Pathol; 105(3):241-254 1981

Terminal deoxynucleotidyl transferase (TdT) is an early T-cell differentiation marker in a number of species, including man. We have demonstrated TdT in the nuclei of cortical thymocytes in paraffin-embedded sections of calf, rat, and human thymus by indirect immunoperoxidase techniques. In addition, these techniques have been used to verify and extend enzyme assay results by detecting TdT in blast cells from 10 patients with convoluted T-cell lymphoma/leukemia, 1 patient with acute granulocytic leukemia, 1 patient with chronic granulocytic leukemia in blast crisis, and 1 patient with non-T non-B acute lymphocytic leukemia. (Author abstract) (49 Refs)

10. POLY ADP-RIBOSYLATION AS A PROSPECTIVE MODULATOR OF CHROMATIN STRUCTURE IN THE HELA CELL NUCLEUS.

Jump DB
Georgetown University Medical Center, Washington, DC
Diss Abstr Int [B]; 42(5):1858-B 1981

Several critical issues dealing with understand.ng the function of poly (ADP-Rib) polymerase in the HeLa nucleus have been addressed: (a) purification and characterization of poly (ADP-Rib) polymerase from HeLa nuclei; (b) determining that

histone modification is most prominent during periods of DNA synthesis; (c) distribution of the enzyme and its acceptor proteins in chromatin with particular emphasis on the elucidation of the association of these proteins with extended regions of chromatin undergoing DNA synthesis; and (d) preliminary evidence indicating that poly ADP-ribosylation may function to promote condensation of modified domains resulting in decreased accessibility of chromatin DNA to nucleases. (no Refs)

11. REGULATION OF RIBONUCLEOTIDE REDUCTASE IN MAMMALIAN CELLS BY CHEMOTHERAPEUTIC AGENTS.

Elford HL, Van't Riet B, Wampler GL, Lin AL, Elford RM
Dept. Biochemistry, MCV/VCU Cancer Center, Medical Coll. Virginia, Richmond, VA, 23298
Adv Enzyme Regul; 19:151-168 1981

The reductive conversion of ribonucleotides to deoxyribonucleotides is a prime target for the development of a cancer chemotherapeutic agent. A new series of inhibitors based on the polyhydroxy or polyamino aromatic ring has been developed. These compounds are effective ribonucleotide reductase inhibitors and possess antitumor activity if there are adjacent hydroxy or amino groups. The most effective enzyme inhibitor, 2,3,4-trihydroxybenzohydroxamic acid, is 145x more effective than hydroxyurea. However, the best antileukemic compound is 3,4-dihydroxybenzohydroxamic acid, which increased the life span of L1210 leukemic mice more than 100%. Structure-activity studies have revealed that the hydroxamic moiety is not essential for activity. The polyhydroxybenzene derivatives reduced the pool sizes of all four deoxynucleotides. This contrasts with the action of hydroxyurea, which depletes only the deoxypurines. The mechanism of inhibition by this group of compounds appears to be related to their ability to trap free radicals, since there is good correspondence between reductase inhibition and free radical destruction. Some naturally occurring cytotoxic and neurological agents which have antineoplastic activity, dopa analogs and phenolic compounds isolated from mushrooms, bear a structural similarity to the new reductase inhibitors. We found that the dopa analogs were also inhibitory to ribonucleotide reductase. Another consequence of polyhydroxybenzoic acid derivative treatment is elevated reductase levels in the cell. Other cell cycle inhibitors that block from late G1 through early G2 also cause an enhanced level of ribonucleotide reductase. However, agents that block in early or mid-G1 or mid or late G2 and mitosis produce lower reductase levels. These data suggest that reductase synthesis is initiated at the G1/S transition point and this enhanced level of activity continues until late S or G2. (Author abstract) (43 Refs)

MECHANISMS OF DNA REPAIR

12. DNA-REPAIR SYNTHESIS IN ATAXIA TELANGIECTASIA LYMPHOBLASTOID CELLS.

Ford MD, Houldsworth J, Lavin MF
Dept. Biochemistry, Univ. Queensland, Brisbane, 4067, Australia
Mutat Res; 84(2):419-427 1981

DNA-repair synthesis was assayed in lymphoblastoid cells from ataxia telangiectasia (AT) patients and from control cells following exposure to gamma-radiation (10-40 kilorad). NaI isopycnic gradient analysis and 3H-thymidine incorporation into acid-precipitable DNA in the presence of hydroxyurea indicated that AT heterozygote, AT homozygote, and control cells had similar capacities for repair of radiation damage. Rate and extent of dose-dependent inhibition of DNA replication by gamma-radiation were similar in the three groups of cells. Lack of difference between control and AT cells in degree of DNA-synthesis inhibition at the radiation doses used is likely due to a pronounced effect on overall DNA synthesis. (34 Refs)

13. DNA REPAIR IN A UV-SENSITIVE MUTANT OF A MOUSE CELL LINE.

Sato K, Setlow RB
Div. Genetics, Natl. Inst. Radiological Sciences, 9-1, Anagawa-4-chome, Chiba-shi 260, Japan
Mutat Res; 84(2):443-455 1981

A UV-sensitive mouse cell mutant was used to examine the contribution of excision repair and post-replication repair to UV-tolerance. UV induced equal numbers of endonuclease-sensitive sites in Raji cells, mouse lymphoma L5178Y cells, and Q31 cells (a UV-sensitive mutant of L5178Y). Raji and L5178Y cells irradiated with 10 Joule/square meter removed 59% and 18% of the sites, respectively, whereas Q31 removed only 3%. Q31 cells were as efficient as the parental cells in post-replication repair. Excision-repair deficiency apparently is responsible for hypermutability and hypersensitivity to UV in Q31 cells. (26 Refs)

14. HOST CELL REACTIVATION OF UV- AND X-RAY-DAMAGED HERPES SIMPLEX VIRUS BY EPSTEIN-BARR VIRUS (EBV)-TRANSFORMED LYMPHOBLASTOID CELL LINES.

Henderson EE, Long WK
Dept. Microbiology and Immunology, Fels Res. Inst., Temple Univ. Sch. Medicine, Philadelphia, PA, 19140
Virology; 115(2):237-248 1981

The efficacy of using an infected centers assay, employing herpes simplex virus-infected, Epstein-Barr virus-transformed lymphoblastoid cell lines (LCLs) as components, to study host cell reactivation has been explored. Herpes simplex virus Type 1 (HSV-1) was shown through the infected centers assay to have detectable but varying ability to lytically infect LCLs established from chromosomal breakage syndromes or closely related genetic disorders. The rate of HSV inactivation by UV irradiation was faster in LCLs established from Cockayne's syndrome than in normal LCLs, and faster still in LCLs established from xeroderma pigmentosum. These results indicate that Cockayne's syndrome, while having what appears to be quantitatively normal levels of UV-induced DNA repair replication, shows decreased ability to host cell reactivate UV-damaged HSV. In direct contrast, x-irradiated HSV showed identical survival when assayed on normal LCLs or LCLs established from ataxia telangiectasia showing increased sensitivity to x-irradiation as measured by colony formation. Through the infected centers assay, it has also been possible to demonstrate low levels of multiplicity reactivation of mutagen-damaged HSV in permanently proliferating LCLs. (Author abstract) (43 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

15. NEW DIMERIC ANALOGUES OF ETHIDIUM: SYNTHESIS, INTERACTION WITH DNA, AND ANTITUMOR ACTIVITY.

Kuhlmann KF, Mosher CW
Bio-Organic Chemistry Lab., SRI International, Menlo Park, CA, 94025
J Med Chem; 24(11):1333-1337 1981

Three new dimeric analogues of ethidium cation in which the monomeric moieties are linked at the 3' positions by alpha,omega-diethers of varying length and composition have been synthesized. The circular dichroism spectra of all three compounds indicate that they double intercalate, and their effects on the thermal helix-coil transition profile of poly(dA-dT) show extremely high affinity for helical DNA, with details of the binding interaction depending on the length and composition of the connecting chain. The ability of the compounds to inhibit nucleic acid synthesis in L1210 cell culture also differed significantly, as did their antitumor effects against P388 leukemia and B16 melanoma. Compound 2, with 10 methylene groups in the connecting chain, is 5-20 x as potent as ethidium against murine P388 leukemia. These results clearly illustrate the advantage gained by incorporating a weak antitumor agent in a double-intercalating analogue. (Author abstract) (22 Refs)

16. PREFERENTIAL ATTACK OF MITOCHONDRIAL DNA BY AFLATOXIN B1 DURING HEPATOCARCINOGENESIS.

Niranjan BG, Bhat NK, Avadhani NG
Lab. Biochemistry, Sch. Veterinary Medicine, Univ. Pennsylvania, Philadelphia, PA, 19104
Science; 215(4528):73-75 1982

Administration of the hepatic carcinogen aflatoxin B1 to experimental animals results in covalent binding to liver mitochondrial DNA at concentrations three to four times higher than nuclear DNA. The concentration of carcinogen adducts in mitochondrial DNA remains unchanged even after 24 hr, possibly because of lack of excision repair. Similarly, mitochondrial transcription and translation remain inhibited up to 24 hr suggesting long-term effects of aflatoxin B1 on the mitochondrial genetic system. (Author abstract) (16 Refs)

17. EFFECT OF N-BUTYRATE ON CELL CYCLE PROGRESSION AND IN SITU CHROMATIN STRUCTURE OF L1210 CELLS.

Darzynkiewicz Z, Traganos F, Xue SB, Melamed MR
Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
Exp Cell Res; 136(2):279-293 1981

In the presence of 1-5 mM n-butyrate, murine leukemic L1210 cells cease proliferation and become arrested in the G1A compartment of the G1 phase. Cells in this compartment are characterized by low RNA content and more condensed chromatin. During unperturbed growth the cell residence times in G1A are of indeterminate duration. The effect of n-butyrate in arresting cells in G1A was concentration-dependent. However, the sensitivity of L1210 cells to this drug was markedly enhanced when cells were treated for longer than one generation (12 hr). Cells arrested in G1A remained viable and when n-butyrate was removed, after a lag period, they resumed progression through the cycle. The effect of n-butyrate on cell progression through various parts of the cycle was studied in a stathmokinetic experiment. DNA in situ in nuclei of n-butyrate-treated cells had lowered (by 2-8 C) stability to thermal denaturation and increased (by 15%) accessibility to DNase I. DNA in situ in the nuclei of n-butyrate-treated cells also showed decreased sensitivity to acid-induced denaturation. Changes in chromatin were seen in all cells, regardless of cell cycle phase, within the first hr after addition of n-butyrate. Mitotic cells, however, reacted to n-butyrate more rapidly than interphase cells. The observed changes in L1210 cells are most likely a consequence of histone modifications (acetylation of inner histones, dephosphorylation of histone H1) induced by n-bu tyrate. (41 Refs)

18. METHYLATION OF LIVER DNA GUANINE IN HYDRAZINE HEPATOTOXICITY: DOSE-RESPONSE AND KINETIC CHARACTERISTICS OF 7-METHYLGUANINE AND O6-METHYLGUANINE FORMATION AND PERSISTENCE IN RATS.

Becker RA, Barrows LR, Shank RC
(c/o Shank), Univ. California Southern Occupational Health Center, Coll. Medicine, Univ. California, Irvine, CA, 92717
Carcinogenesis; 2(11):1181-1188 1981

Fischer 344 or Sprague Dawley rats were fasted overnight and given orally 30-90 mg hydrazine/kg body wt. The presence of 7-methylguanine and O6-methylguanine in liver DNA was demonstrated 5 and 24 hr after hydrazine administration using two different analytical techniques. Methylation levels changed little with dose except for the highest dose (ca LD50) at which the levels doubled. In a time-response study, rats were given 90 mg hydrazine/kg body wt and killed 0.25 to 96 hr later. Both 7-methylguanine and O6-methylguanine were detected quantitatively in liver DNA from rats as early as 15 min after hydrazine administration. After maximum levels of methylguanines had formed, 7-methylguanine was removed from DNA at a rate of approx 50% in 47 hr; the half-life of O6-methylguanine in liver DNA was approx 13 hr. Three or four, but not one or two, daily administrations of 3 mg hydrazine/kg body wt also produced detectable levels of 7-methylguanine in rat liver DNA. Neither 7-methylguanine or O6-methylguanine was detected in comparable amounts of liver DNA from control animals. The study confirms the observation that hydrazine administration results in the formation of methylated guanines in liver DNA. (Author abstract) (40 Refs)

* U. S. GOVERNMENT PRINTING OFFICE : 1982— 361-947/117

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MECHANISMS OF DNA REPLICATION

1. A TEMPERATURE-SENSITIVE MUTANT ISOLATED FROM MOUSE FM3A CELLS DEFECTIVE IN DNA REPLICATION AT A NON-PERMISSIVE TEMPERATURE.

Hyodo M, Suzuki K
Dept. Molecular Biology, Tokai Univ. Sch. Medicine, Boseidai, Isehara-shi, Kanagawa-Ken 259-11, Japan
Exp Cell Res; 137(1):31-38 1982

Ts-131b, one of the temperature-sensitive (ts) mutants isolated from mouse FM3A cells, was found to be defective in DNA replication at a non-permissive temperature. After the cells were transfered to 39.5 C, the cell number increased by only 10% and the rate of incorporation of precursors into cellular DNA decreased rapidly. Cell cycle analysis by a flow cytometric method with the cells incubated at 39.5 C revealed that progression of the cells through the S phase was inhibited and most of the cells were arrested in the S phase. To study

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the defect in DNA replication of this ts-mutant at 39.5 C, DNA-fiber autoradiography was performed to measure the rate of DNA-chain elongation. The results showed that the rate of DNA-chain elongation was decreased at 6 hr after the temperature shift. However, since the decrease in the rate of DNA-chain elongation was not sufficient to account for the decrease in the rate of incorporation of the precursors, it was suggested that there was also a decrease in the rate of initiation of DNA replication at some of the replicon origins. (Author abstract) (31 Refs)

2. RESTRICTED HYDROLYSIS OF NEWLY REPLICATED DNA IN ISOLATED NUCLEI FROM YOSHIDA SARCOMA CELLS.

Akiyoshi H
Dept. Enzyme Physiology, Inst. Enzyme Res., Sch. Medicine, Tokushima Univ., Kuramoto-cho, Tokushima, Tokushima 770, Japan
J Biochem (Tokyo); 90(6):1597-1601 1981

The acid solubility of radioactive nuclear DNA was examined under various conditions using isolated nuclei from [3H]thymidine-labeled Yoshida sarcoma cells. Upon incubation at 37 C, acid-soluble radioactivity increased rapidly in 30 min, and the increase continued gradually during 4 hr incubation. The maximal hydrolysis was obtained at pH 7-7.5 in the presence of 0.25 mM CoCl2. MgCl2 (2mM) replaced CoCl2 with a 30% reduced activity. The ratio of hydrolyzed to the total radioactive DNA increased in proportion to shortening of the pulse labeling times; 7% of the ratio in 8 min pulse-labeled nuclei increased to 18% in 2 min pulse-labeled and 37% in 1 min pulse-labeled nuclei. However, only 2% of the labeled DNA was hydrolyzed in nuclei isolated from cells labeled for 1 min and chased for 20 min in the presence of excess thymidine. Significant inhibition was observed upon addition of guanosine 5'-monophosphate (GMP), 6-mercaptopurine riboside 5'-phosphate, or deoxyribonucleoside triphosphates. The findings showed that divalent cation-dependent DNA hydrolysis was restricted to newly replicated DNA. (Author abstract) (20 Refs)

EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

3. MICROTUBULE STABILIZATION BY TAXOL INHIBITS INITIATION OF DNA SYNTHESIS BY THROMBIN AND BY EPIDERMAL GROWTH FACTOR.

Crossin KL, Carney DH
Dept. Human Biological Chemistry and Genetics, Div. Biochemistry, Univ. Texas Medical Branch, Galveston, TX, 77550
Cell; 27(2):341-350 1981

Several observations have suggested that microtubule depolymerization might act as a regulator of cell proliferation. To determine whether microtubule depolymerization is required for growth-factor-induced initiation of DNA synthesis, we treated serum-free cultures of mouse embryo cells with taxol to stabilize their microtubules and measured the initiation of DNA synthesis by thrombin and epidermal growth factor (EGF). Pretreatment of quiescent cultures of mouse embryo cells with 10 ug/ml ta xol inhibited up to 60% of the thrombin-stimulated and 47% of the EGF-stimulated DNA synthesis. This inhibition was dose-dependent for taxol concentrations from 0.3 to 20 ug/ml. Control experiments showed that taxol did not simply affect uptake of nucleotides, glucose or amino acids, nor did it nonspecifically affect protein synthesis, cell morphology or cell viability. Taxol did not affect binding and internalization of 125I-thrombin or 125I-EGF indicating that the drug does not alter receptor number, affinity or distribution after growth factor binding. Taxol also did not affect the proteolytic activity of thrombin. Thus it appears that the inhibitory effects of toxol are mediated by a direct effect of taxol on microtubules. To determine at which point microtubule stabilization was interrupting the initiation signal, we added taxol at various times after addition of thrombin or EGF. Taxol addition during the first 8 hr after growth factor addition inhibited initiation, but after 8 hr had little if any effect. These results confirm that taxol was not nonspecifically affecting transport or metabolism required for DNA synthesis and indicate that thrombin and EGF may initiate cell proliferation through a gradual microtubule depolymerization or rearrangement that is necessary to commit cells to a replicative cycle. (Author abstract) (48 Refs)

4. A NUCLEAR ANTIGEN ASSOCIATED WITH CELL PROLIFERATION AND BLAST TRANSFORMATION. ITS DISTRIBUTION IN SYNCHRONIZED CELLS.

Takasaki Y, Deng JS, Tan EM
Div. Rheumatic Diseases, Dept. Medicine, Univ. Colorado Sch. Medicine, Denver, CO, 80262
J Exp Med; 154(6):1899-1909 1981

The time relationship of nuclear antigen present in proliferating cells (PCNA) to synthesis of DNA, RNA, and protein during the cell cycle was examined. Two sera (EB and MN) from patients with systemic lupus erythematosus contained precipitating anti-PCNA antibody along with antibodies to other nuclear antigens. Insolubilized rabbit kidney extract was used as an immunoadsorbant; precipitating antibodies to other nuclear antigens were removed with the exception of anti-PCNA. WiL-2 diploid human B lymphocytes were examined with EB-absorbed serum at different phases of synchronized growth. At G0, immediately after release from density-dependent arrest, PCNA were present as speckled nucleoplasmic staining. At 12 hr, nucleolar staining and speckled nucleoplasmic staining were evident. At 20 hr, nucleolar staining had disappeared from most cells, but speckled nucleoplasmic staining was strong. In mitosis, staining was more intense in areas peripheral to chromosome condensation, but radiating strands of staining could be seen within the region of chromosome condensation. In WiL-2 cells, nucleolar staining predominated in a period which overlapped late G1 and early S, while in phytohemagglutinin-stimulated lymphocytes, nucleolar stain ing was the dominant pattern well before DNA synthesis occurred. Results demonstrate that the relationship of PCNA to proliferation and blast transformation may be associated with events related to DNA synthesis in the cells. (14 Refs)

5. MELPHALAN PREVENTS THE FORMATION OF DOUBLE-STRANDED DNA REPLICATION INTERMEDIATES IN HUMAN MALIGNANT MELANOMA CELLS.

Lonn U, Lonn S
Dept. Histology, Karolinska Institutet, P.O. Box 60400, S-104 01 Stockholm, Sweden
Carcinogenesis; 2(12):1311-1316 1981

When human melanoma cells are lysed in a neutral buffer they release from the chromosomes double-stranded DNA replication intermediates. We have now examined the effect of a bifunctional alkylator (melphalan) on the formation of these in-

termediates. After treatment for 25 min there is little or no release of double-stranded DNA intermediates from the chromosomes. When the cells are washed free of the drug and cultivated further there is a resumed formation of the DNA intermediates. This is paralleled by the finding that the cells, when the drug is removed, after a time-lag, regain the capacity to proliferate. (Author abstract) (7 Refs)

6. INHIBITORY EFFECT OF BOVINE SEMINAL RIBONUCLEASE ON ACTIVATED LYMPHOCYTE LYMPHOBLASTOID CELL LINES IN VITRO.

Soucek J, Matousek J, Chudomel V, Lindnerova G
Inst. Haematology and Blood Transfusion, 128 20 Praha, Czechoslovakia
Folia Biol (Praha); 27(5):334-345 1981

The effects of bovine seminal ribonuclease (AS RNase) and its four modified derivatives on pokeweed mitogen (PWM)-stimulated human lymphocytes, on mixed lymphocyte cultures, and on growth of four lymphoblastoid cell lines were examined. Native AS RNase, isolated as a dimer from bovine seminal vesical fluid, was used to prepare the carboxymethylated, succinylated, oxidized, and reduced forms of the enzyme. Native AS RNase exerted the strongest suppressive effect on phytohemagglutinin (PHA)-, PWM-, and mixed lymphocyte reaction-stimulated lymphocytes. At 5 and 100 ug/ml, AS RNase inhibited lymphocyte stimulation by 40 and 95%, respectively. The inhibitory effect persisted in the presence of a 20-fold increase in PHA. Simultaneous addition of AS RNase and (3H)thymidine to transformed lymphocytes did not affect thymidine uptake. AS RNase also had the strongest inhibitory effect on growth, viability, and DNA in the four lymphoblastoid lines tested. AS RNase ***decreased DNA synthesis*** in Molt-3 and UHKT-2 cells, with a decrease in live cell count compared to controls. Immunosuppressive effects of AS RNase suggest that the compound is one component of seminal plasma which may take part in suppression of immune responses of the female reproductive tract to spermatozoa. (21 Refs)

7. STIMULATION OF DNA SYNTHESIS AND CELL PROLIFERATION IN THE LIVER OF RATS FED A CHOLINE-DEVOID DIET AND THEIR SUPPRESSION BY PHENOBARBITAL.

Abanobi SE, Lombardi B, Shinozuka H
Dept. Pathology, Univ. Pittsburgh Sch. Medicine, Pittsburgh, PA, 15261
Cancer Res; 42(2):412-415 1982

Feeding of a choline-devoid (CD) diet and dietary administration of phenobarbital (PHB) are efficient promoters of liver carcinogenesis in the rat. Furthermore, inclusion of PHB in a CD diet results in a synergistic effect, inasmuch as the promoting action of their combination is greater than the sum of those exerted by either agent alone. To investigate the mechanism(s) of action of the two promoters, liver DNA synthesis and liver cell proliferation were studied in rats fed a CD diet, a choline-supplemented diet, or the same diets to which 0.06% PHB was added. DNA synthesis was determined by [3H]thymidine incorporation into DNA and autoradiography, and cell proliferation was determined by mitosis counts. Feeding the CD diet caused an increase of both DNA synthesis and cell proliferation over those present in rats fed the choline-supplemented diet. Inclusion of PHB in the CD diet, on the other hand, inhibited DNA synthesis and cell proliferation. These results indicate that stimulation of cell proliferation per se may not be a sufficient condition for an agent to act as a promoter of liver carcinogenesis. (Author abstract) (32 Refs)

8. COMPARATIVE TOXICITY OF CHEMOTHERAPY FOLLOWING PARTIAL HEPATECTOMY.

Grosfeld JL, Weber TR, Baehner rL, Hays DM, Vos A, Filler RM
Dept. Surgery, J.W. Riley Hosp. for Children (K-21), 1100 West Michigan St., Indianapolis, IN, 46223
J Pediatr Surg; 16(6):950-954 1981

Effects of commonly employed chemotherapeutic agents on regenerating liver was studied in 200 Sprague Dawley rats. After 70% hepatectomy, animals were divided into four groups: Group I consisted of untreated controls, Group II received actinomycin D (AMD) at 150 ug/kg iv, Group III received vincristine (VCR) at 0.2 mg/kg iv, and Group IV received adriamycin (ADR) at 30 mg/kg iv. Thymidine incorporation into DNA and thymidine degradation to carbon dioxide were measured in liver slices. Peak DNA levels (8.2 x 10(3) cpm) and lowest CO2 levels were seen at 24 hr in control animals. Group III animals had values for both parameters which were similar to those in controls. Thymidine incorporation into DNA in Group IV (ADR) animals was 5.1 x 10(3) cpm; incorporation was lowest in Group II animals (AMD), at 3.78 x 10(3) cpm. Liver wt in ADR and AMD-treated animals was significantly lower than in controls or VCR-treated animals. Results indicate that while AMD and ADR severely ***inhibit DNA synthesis*** in regenerating liver, VCR is relatively benign. (11 Refs)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

9. ALTERED SENSITIVITY TO 1-BETA-D-ARABINOFURANOSYLCYTOSINE 5'-TRIPHOSPHATE OF DNA POLYMERASE ALPHA FROM LEUKEMIC BLASTS OF ACUTE LYMPHOBLASTIC LEUKEMIA.

Tanaka M, Yoshida S
Nagoya Natl. Hosp., Hematological Disease Center, Sannomaru, Nagoya, Japan
Cancer Res; 42(2):649-653 1982

DNA polymerase alpha from the leukemic cells of acute lymphoblastic leukemia (ALL) was found to be more resistant to the inhibition by 1-beta-D-arabinofuranosylcytosine 5'-triphosphate than that from acute myeloblastic leukemia (AML). Apparent Ki values for 1-beta-D-arabinofuranosylcytosine 5'-triphosphate of DNA polymerase alpha from eight patients with ALL [26.7 +-7.1 (S.D.) uM] were 5x higher than those from nine patients with AML [5.2 +-1.3 uM]. In contrast, apparent Km values for a normal substrate deoxycytidine 5'-triphosphate of DNA polymerase alpha preparations from either AML and ALL were almost identical (9.4 to 10.9 uM). Likewise, apparent Ki values for another arabinoside analog, 9-beta-arabinofuranosyladenine 5'-triphosphate, of DNA polymerase alpha from blasts of seven patients with ALL (16.9 +-6.9 uM) were significantly higher than those from four patients with AML (3.8 +-0.5 uM). These results indicate that DNA polymerase alpha from all blast cells has a decreased affinity to the arabinoside analogs of deoxynucleotide triphosphate. The sensitivity of DNA polymerase alpha of blast cells to 1-beta-arabinofuranosylcytosine 5'-triphosphate may be one of the determinants of the clinical response to 1-beta-D-arabinofuranosylcytosine treatment. (Author abstract) (14 Refs)

10. INTERACTION OF 5'-PHOSPHO-1-BETA-D-ARABINOFURANOSYL-5-SUBSTITUTED URACILS WITH THYMIDYLATE SYNTHETASE: MECHANISM-BASED INHIBITION BY ARA-FUMP (MEETING ABSTRACT).

Wataya Y, Saneyoshi M, Ueda T, Santi DV, Nakayama C
Okayama University, Okayama, Japan
Proceedings of the Japanese Cancer Association, 40th Annual Meeting, October 1981, Sapporo, Japan. The Association 415 pp., 1981.

A number of 5'-phospho-1-beta-D-arbinosyl-5-substituted uracils (ara-UMP's) have been examined as inhibitors of Thymidylate (dTMP) synthetase. As reversible inhibitors, all were substantially less potent than their 2'-deoxyribosyl counterparts. In the presence of 5,10-methylenetetrahydrofolate (CH(2)-H4 folate), ara-5-fluorouracil monophosphate (FUMP) caused a first-order, time-dependent inactivation of the enzyme. At 0 degrees, kinetic studies indicated a reversible Kd of 3.6 uM for the ara-FUMP-CH(2)-H4 folate complex, and K equals 0.22 per min for the subsequent inactivation. Spectral studies of the complex and its behavior towards protein denaturants demonstrate that its structure and stoichiometry are directly analogous to those which have previously been described for fluorodeoxyuridylate (FdUMP). The significance of this finding with regard to pro-drugs of ara-FU and potential of ara-FU as a chemotherapeutic agent are d iscussed. (no Refs)

11. EFFECT OF HYPERTHERMIA ON CHO DNA POLYMERASES ALPHA AND BETA.

Spiro IJ, Denman DL, Dewey WC
Dept. Oncology, George Washington Univ. Medical Center, Washington, DC, 20037
Radiat Res; 89(1):134-149 1982

Loss of polymerase beta (pol-B) activity assayed in Chinese hamster ovary (CHO) cell extracts following thermal treatment of whole cells was examined. Pol-B activity decreased progressively as the treatment temperature was increased from 42.2 to 45.5 C. At temperatures of 43 C and greater, activity loss was exponential as the duration of treatment was lengthened. The heat sensitivities of pol-B and polymerase alpha (pol-A) were compared at 45.5, 43.5, and 42.3 C. At the two higher temperatures, pol-A was considerably more heat stable than pol-B with greater than 10% of the pol-A activity remaining after a 35-min heat dose at 45.5 C. The heat sensitivities of pol-A and pol-B were identical when cells were treated at 42.3 C. Pol-B activity was shown to be more sensitive to combinations of heat and procaine-HCl than to heat alone. The heat inactivation rates for synchronous G1-and S-phase cells were the same and identical to rates for asynchronous cells, although the absolute level of pol-B is higher in S-phase cells than in G1 cells. No diffusible factor was produced by heated CHO cells which could cause inhibition of pol-B by transfer to the unheated sample. The return of pol-B activity following heating is described. Inhibition of unscheduled DNA synthesis (UDS) correlated very well with loss of pol-B activity. The data demonstrate that the loss of intracellular pol-B activity correlates with heat killing, heat radiosensitization, and a reduction in repair capability as measured by UDS. (64 Refs)

12. DIRECT PHOTOAFFINITY LABELING OF AN ALLOSTERIC SITE ON SUBUNIT PROTEIN M1 OF MOUSE RIBONUCLEOTIDE REDUCTASE BY DTTP.

Eriksson S, Caras IW, Martin DW
Dept. Biochemistry, Karolinska Inst., Stockholm, Sweden
Proc Natl Acad Sci USA; 79(1):81-85 1982

The protein M1 subunit of ribonucleotide reductase contains at least two allosteric nucleotide binding sites that control the capacity of the enzyme to reduce ribonucleotides to the deoxyribonucleotides required for DNA synthesis. Direct photoaffinity labeling of partially purified protein M1 from mouse T-lymphoma (S49) cells was observed after UV irradiation in the presence of dTTP at 0 C. The relative molar incorporation of nucleotide per subunit was 4-8%. Competition experiments showed that the dTTP was bound to an allosteric domain genetically and kinetically defined as the substrate specificity site of the enzyme. An altered protein M1 isolated from a thymidine-resistant mutant cell line showed significantly decreased photoincorporation of dTTP, consistent with the fact that its CDP reductase acti vity is resistant to feedback inhibition by dTTP. Specific photolabeling of several other proteins with pyrimidine and purine nucleotides was also found, indicating the general usefulness of direct photoaffinity labeling in the study of enzymes involved in nucleotide and nucleic acid metabolism. (Author abstract) (11 Refs)

MECHANISMS OF DNA REPAIR

13. INTERACTION OF HUMAN AND CHICK DNA REPAIR FUNCTIONS IN UV-IRRADIATED XERODERMA PIGMENTOSUM-CHICK ERYTHROCYTE HETEROKARYONS.

Bootsma D, Keijzer W, Van Der Veer E, Rainaldi G, De Weerd-Kastelein EA
Dept. Cell Biology and Genetics, Erasmus Univ. Rotterdam, 3000 DR Rotterdam, The Netherlands,
Exp Cell Res; 137(1):181-189 1982

Fusion of chick erythrocy .s with human primary fibroblasts results in the formation of heterokaryons in which the inactive chick nuclei become reactivated. The expression of chick DNA repair functions was investigated by the analysis of the DNA repair capacity after exposure to ultraviolet (UV) irradiation of such heterokaryons obtained after fusion of chick erythrocytes with normal human or xeroderma pigmentosum (XP) cells of complementation groups A, B, C, and D. Unscheduled DNA synthesis (UDS) in normal human nuclei in these heterokaryons is suppressed during the first 2-4 days after fusion. The extent and duration of this suppression is positively correlated with the number of chick nuclei in the heterokaryons. Suppression is absent in heterokaryons obtained after fusion of chicken embryonic fibroblasts with XP cells (complementation group A and C). Restoration of DNA repair synthesis is found after fusion in XP nuclei of all complementation groups studied. It occurs rapidly in XP group A nuclei, starting one day after fusion and reaching near normal human levels after 5-8 days. In nuclei of the B, C and D group increased levels of UDS are found 5 days after fusion. At 8 days after fusion the UDS level is about 50% of that found in normal human nuclei. The pattern of UDS observed in the chick nuclei parallels that of the human counterpart in the fusion. A fast complementation pattern is also observed in chick fibroblast-XP group. A heterokaryons resulting within 24 hr in a UDS level comparable with that in chick fibroblast-normal human heterokaryons. In heterokaryons obtained after fusion of chick fibroblasts with XP group C cells UDS remains at the level of chick cells. These data suggest that reactivation of chick erythrocyte nuclei results in expression of repair functions which are able to complement the defects in the XP complementation groups A, B, C and D. (Author abstract) (15 Refs)

14. AN APPARENT CORRELATION BETWEEN THE INHIBITION OF INDUCED ORNITHINE DECARBOXYLASE ACTIVITY BY GAMMA RADIATION AND THE CAPACITY FOR DNA REPAIR SYNTHESIS IN NORMAL AND ATAXIA TELANGIECTASIA HUMAN FIBROBLASTS: NO CORRELATION WITH CELL SURVIVAL.

Ben-Hur E, Kol R, Heimer YM, Shiloh Y, Tabor E, Becker Y
Dept. Radiobiology, Nuclear Res. Centre-Negev, P.O. Box 9001, Beer-Sheva, Israel
Radiat Environ Biophys; 20(1):21-28 1981

The effects of gamma radiation on induced ornithine decarboxylase (ODC) activity, DNA repair synthesis (DRS), and cell survival were studied in vitro in fibroblasts from normal individuals and patients with ataxia telangiectasia (AT). ODC activity in nonirradiated human fibroblasts was induced after a lag period of 2-3 hr by addition of fresh medium and reached a peak at 7-8 hr. A dose of 108 kilorad (krad) gamma radiation before induction substantially reduced ODC activity in normal cells of strain F107 and AT cells of strains F182 and F184. Cells of strain F196, from an apparently normal individual, showed much greater sensitivity than the other strains. F107 and AT cells showed an inhibition of DRS paralleling the decrease in induction of ODC. F196 cells showed a greater reduction in DRS capacity paralleling their greater inhibition of ODC induction. F107 and F196 showed similar cell survival curves after gamma irradiation (D37 values being 150 +-7 and 135 +-6 rad, respectively). AT cells were more sensitive than normal cells (D37 being 60 +-2 rad for F182 and 45 +-2 rad for 184). Apparently sensitivity of ODC induction and DRS are related, possibly in a causal manner, in normal and AT human fibroblast; cell survival is apparently not related to either ODC induction or DRS. (19 Refs)

15. HUMAN MONOCYTES AND LYMPHOCYTES DIFFER IN THE RATE OF DNA REPAIR SYNTHESIS AND VIABILITY AFTER EXPOSURE TO NITROGEN MUSTARD.

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Acta Med Scand; 210(5):381-387 1981

Unscheduled DNA synthesis was studied in human lymphocytes and monocytes after treatment with nitrogen mustard, methyl-methane-sulfonate and ultraviolet irradiation. The unscheduled DNA synthesis was about 4x higher in monocytes than in lymphocytes after treatment with nitrogen mustard. Treatment with methyl-methane-sulfonate or ultraviolet irradiation confirmed the higher capacity for unscheduled DNA synthesis in the monocytes. When Ficoll-Isopaque-isolated mononuclear cells were exposed to various doses of nitrogen mustard and incubated for different periods, a dose- and time-dependent increase in trypan blue uptake was found. Furthermore, a corresponding dose- and time-dependent decrease was noted in 3H-uridine incorporation. Since trypan blue uptake will not enable differentiation between monocytes and lymphocytes appearing as a mixed cell population, 3H-uridine flash labeling index was studied after exposure to nitrogen mustard. There was a dose- and time-dependent decrease in 3H-uridine labeling index, suggesting a correlation between the absence of 3H-uridine incorporation and increase in trypan blue upta ke. When compared to monocytes, the lymphocytes were more affected in this respect after exposure to nitrogen mustard. The possibility that differences in cytotoxic effect may be related to DNA repair mechanisms is discussed. (Author abstract) (34 Refs)

16. ALTERED DNA REPAIR IN FIBROBLASTS FROM AGED RATS.

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Dept. Toxicology, Abbott Lab., North Chicago, IL, 60064
Gerontology; 27(6):306-312 1981

The role of DNA repair in the aging process was studied using fibroblast cultures derived from rats at the beginning (3 wk) and the end (2 yr) of their potential lifespan. The effect of treatment of the cultures with the DNA-damaging agent, ethylnitrosourea, on the integrity of the pre-existing, parental DNA and on de novo DNA synthesis was determined using the alkaline elution method. It was found that drug treatment resulted in lower levels of fragmentation of parental DNA and in decreased fractions of short daughter strands in cells derived from old relative to young animals. These results indicate an alteration of the processing of DNA damage in cells derived from aged rats. (Author abstract) (37 Refs)

17. ISOLATION AND PARTIAL CHARACTERIZATION OF MUTAGEN-SENSITIVE AND DNA REPAIR MUTANTS OF CHINESE HAMSTER FIBROBLASTS.

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Environ Mutagen; 3(1):53-64 1981

Isolation nd partial characterization of several mutants of Chinese hamster cells, which are affected in their ability to repair DNA damage or in their sensitivity to radiation or chemical mutations, are discussed. Cells of a subclone of the V79 aneuploid cell line were mutagenized by combined treatment with 5-bromodeoxyuridine and blacklight, followed by UV irradiation. 3H-thymidine was incorporated in repair-proficient cells at high temperature (38.5 C) following UV damage. Cells were held in the cold (4.0 C) to induce radioactive thymidine killing. Repair-deficient and UV-sensitive cells which had survived and formed colonies at low temperature (34.0 C) were tested for recovery. Seventy-two surviving colonies were isolated from 2 x 10(7) cells plated for selection. Four colonies were selected for extensive study. Colony UVs-7 was slightly more sensitive to UV, but not sensitive to x-rays or N-acetoxy-2-acetylaminofluorene (NAc-AAF); this mutant exhibited a highly reduced level of unscheduled DNA synthesis (UDS), as compared to the parental line. UVs-40 and UVs-44 line colonies were sensitive to UV, x-ray, N-methyl-N-nitro-N-nitrosoguanidine, and NAc-AAF, but exhibited normal UDS. Line UVr-23 had enhanced UDS, was resistant to UV, but exhibited no difference in sensitivity to x-ray or NAc-AAF. These four mutants are all stable, and may be useful for the study of mammalian DNA repair processes and me chanisms of mutagenesis. (38 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

18. CELL ONCOGENES ARE LOCATED ON THE LARGE MICROCHROMOSOMES IN CHICKEN CELLS.

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Virology; 116(2):654-657 1982

The chromosomal locations of c-myc and c-myb, the cell homologs of the transforming genes present in myelocytomatosis virus 29, MC29, and avian myeloblastosis virus, AMV, respectively, were determined in White Leghorn chickens using in situ hybridization. Nucleic acid probes containing MC29 and AMV-RNAs were used to localize both c-myc and c-myb to a subset of large microchromosomes. The chromosomal location of c-myc was confirmed using nucleic acid probes containing RNA from MH-2, which also contains a myc-like sequence, and cloned DNA containing the chicken c-myc gene. In conjunction with our previous results localizing c-src and c-erb, we have demonstrated that several cell oncogene homologs appear to be localized on a subset of large microchromosomes. (Author abstract) (18 Refs)

19. THREE SEGMENTS FROM THE MONKEY GENOME THAT HYBRIDIZE TO SIMIAN VIRUS 40 HAVE COMMON STRUCTURAL ELEMENTS.

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Mol Cell Biol; 1(12):1061-1068 1981

Recently, three cloned segments were isolated from a monkey genomic library. The segments hybridizing to the HindIII-C fragment of the DNA virus, simian virus 40 (SV40) are discussed. The primary structure of one segment has been reported. As a further step in analyzing the relationship between the SV40-hybridizing segments and SV40 itself, the sequence and genomic arrangements of the other two cloned segments was determined by previously described methods, and a comparison was made of all three. The SV40 hybridizing region in each segment was limited to several hundred base pairs. All segments contained multiple and disconnected sequences homologous to the region of SV40 directly surrounding the viral replication origin. While the number and arrangement of the homologous sequences was different in the three segments, each had the following features in common: (1) each contained multiple copies of the sequence GGGCGGPuPu, which also appeared 6x near the origin of SV40, (2) each contained several strong homologies to the central dyad symmetry of SV40, and (3) each contained a long internal repeat, as did the origin region of SV40. These three SV40 hybridizing segments are members of a larger family of genomic sequences that hybridize well to each, but not necessarily to SV40. (18 Refs)

20. ORGANIZATION AND EXPRESSION OF ENDOGENOUS VIRUS-LIKE (VL30) DNA SEQUENCES IN NONTRANSFORMED AND CHEMICALLY TRANSFORMED MOUSE EMBRYO CELLS IN CULTURE.

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Cancer Res; 42(2):569-576 1982

A cloned mouse DNA fragment containing an endogenous 'virus-like' DNA (VL30 DNA) sequence was identified by virtue of its ability to hybridize to the virus-like RNA component of mixed-pseudotype AKR-murine leukemia virus virions, its lack of detectable sequence homology with cloned AKR-murine leukemia virus DNA, and its hybridization to a 5.6 kilobase pair (30S) cellular polyadenylic acid [poly(A)]-containing RNA species. Restriction enzyme mapping of the cloned mouse fragment revealed the presence of a 5-to 6-kilobase pair VL30 DNA segment flanked by non-VL30 segments of approx 7 and 0.3 kilobase pairs. Southern blot analysis of VL30 DNA sequence organization in the DNA of two nontransformed mouse cell lines (AKR-2B, C3H/10T1/2) and two chemically transformed derivatives (AKR-MCA, C3H/MCA-58) revealed 15 to 20 bands organized in an apparent strain-specific pattern. Within a given strain, however, no differences were detectable between the nontransformed cells and their chemically transformed counterparts. The expression of VL30 genes in the above cell lines was assayed by hybridization of 32P-labeled poly(A)-containing polysomal RNA to several internal restriction fragments derived from the cloned VL30 DNA sequence. The level of VL30 RNA was enhanced approx 10-fold in both chemically transformed cell lines as compared to the nontransformed cell lines (under normal growth conditions). In addition, nontransformed AKR-2B cells maintained in the presence of purified epidermal growth factor exhibited similarly enhanced levels of VL30 RNA sequences in polysomal RNA. Since these cells display several growth characteristics of transformed cells but, in an epidermal growth factor-dependent and completely reversible fashion, these data suggest that the expression of VL30 genes is not simply incidental to chemically transformed cells but may be related to alterations in growth control. (Author abstract) (34 Refs)

21. LONG TERMINAL REPEAT OF MURINE RETROVIRAL DNAS: SEQUENCE ANALYSIS, HOST-PROVIRAL JUNCTIONS, AND PREINTEGRATION SITE.

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J Virol; 41(2):542-556 1982

The nucleotide sequence of the long terminal repeat (LTR) of three murine retroviral DNAs has been determined. The data indicate that the U5 region (sequences originating from the 5' end of the genome) of various LTRs is more conserved than the U3 region (sequences from the 3' end of the genome). The location and sequence of the control elements such as the 5' cap, 'TATA-like' sequences, 'CCAAT-box,' and presumptive polyadenylic acid addition signal AATAAA in the various LTRs are nearly identica l. Some murine retroviral DNAs contain a duplication of sequences within the LTR ranging in size from 58 to 100 base pairs. A variant of molecularly cloned Moloney murine sarcoma virus DNA in which one of the two LTRs integrated into the viral DNA was also analyzed. A four-base-pair duplication was generated at the site of integration of LTR in the viral DNA. The host-viral junction of two molecularly cloned AKR-murine leukemia virus DNAs (clones 623 and 614) was determined. In the case of AKR-623 DNA, a three- or four-base-pair direct repeat of cellular sequences flanking the viral DNA was observed. However, AKR-614 DNA contained a five-base-pair repeat of cellular sequences. The nucleotide sequence of the preintegration site of AKR-623 DNA revealed that the cellular sequences duplicated during integration are present only once. Finally, a striking homology between the sequences flanking the preintegration site and viral LTRs was observed. (Author abstract) (44 Refs)

22. INTERACTIONS OF A FRAGMENT OF BLEOMYCIN WITH DEOXYRIBODINUCLEOTIDES: NUCLEAR MAGNETIC RESONANCE STUDIES.

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Biochemistry; 20(26):7589-7598 1981

Proton NMR spectroscopy was used to establish certain geometrical parameters of the complexes formed between N-(3-aminopropyl)-2'-(2-acetamidoethyl)-2,4'-bithiazole 4-carboxamide hydrochloride (BLMF), a fragment of bleomycin, and various deoxyribodinucleotides. All proton resonances in these compounds have been assigned; chemical shifts were recorded as functions of their concentration. In the complex formed between BLMF and pdG-dC, chemical shifts of the bithiazole protons (measured with respect to values extrapolated to infinite dilution) were displaced upfield by 0.4 ppm. Other proton resonances of BLMF were shifted upfield but to a lesser extent. After corrections are made for self-stacking, maximum values for induced chemical shifts of the bithiazole protons are reached at a dinucleotide/BLMF ratio of 2. Coupling sums for dinucleotides (12.5-13.7 Hz) were unchanged following complexation, suggesting that there is no marked change in sugar conformation when BLMF is bound. On the basis of these results and of molecular model building studies, we propose a three-dimensional structure of a BLMF:pdG-dC complex in which the thiazole rings are intercalated in the duplex and stack preferentially on the purines. Projected on the same plane, the horizontal axis connecting the center of both bithiazole rings in this configuration superimposes on the axis connecting the centers of the purine bases. In this complex, both thiazole protons extend into the minor groove and the positively charged terminal amine binds to the negatively charged phosphate group of DNA. (Author abstract) (42 Refs)

23. STRAND SCISSION OF DEOXYRIBONUCLEIC ACID BY NEOCARZINOSTATIN, AUROMOMYCIN, AND BLEOMYCIN: STUDIES ON BASE RELEASE AND NUCLEOTIDE SEQUENCE SPECIFICITY.

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Biochemistry; 20(26):7599-7606 1981

The nucleotide sequence specificity of neocarzinostatin (NCS), auromomycin (AUR), bleomycin (Blm), phleomycin (Phlm), and tallysomycin (Tlm) has been determined by using these antibiotics and their associated chromophores to create strand scissions in end-labeled restriction fragments of DNA and then determining the base sequence of the oligonucleotides formed. NCS and the NCS chromophore induce similar patterns of cleavage in DNA fragments labeled at the 5' terminus. The pattern produced by the AUR chromophore also resembles that of its holoantibiotic. Dithiothreitol enhances the rate of cleavage of DNA by the AUR chromophore but does not alter the sequence specificity. The results suggest that the polypeptide component of AUR and NCS serves primarily as a carrier for the chromophore. When tested with a fragment labeled at the 3' terminus, the products of NCS and AUR cleavage do not display the patterns of chemically produced oligonucleotides cleaved at phosphodiester bonds, suggesting that the 5' terminus is modified by a sugar fragment. NCS primarily attacks thymine (75% of the total bases attacked) and, to a lesser extent, adenine (19%) and cytosine (6%). AUR preferentially attacks guanine (67% of total bases), while attacking less often thymine (24%) and adenine (9%). Bleomycin and its analogues preferentially cleave purine-pyrimidine (5' to 3') and pyrimidine-pyrimidine (3' to 5') sequences. All (5' to 3') GT and GC sequences were cleaved. Phlm G and Phlm-Pep are less active than bleomycin toward purines while Tlm was more active. The patterns of cleavage produced by Blm A2 and Glm B6 are similar, while those produced by Phlm-Pep, Phlm G, Blm-B1', and Blm-Pep resemble one another. The cleavage pattern of Tlm shows quantitative differences from the other analogues tested. Differences between bleomycin and its analogues may be related to structural differences in these molecules. (Author abstract) (36 Refs)

24. INTERACTION OF THE ANTITUMOUR DRUG 4'-(9-ACRIDINYLAMINO)-METHANESULFON-M-ANISIDINE.HCL (M-AMSA) WITH NUCLEIC ACIDS.

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Nucleic Acids Res; 9(24):6959-6973 1981

The interaction of AMSA with nucleic acids was studied by several techniques. Melting temperature and CD studies equally suggest that AMSA-binding is interfering with the secondary structure of DNA. An overlap by two mechanisms of binding seems to exist. Based on the CD measurements at low drug concentration intercalation is the most likely way of binding. At higher drug concentration stacking interaction predominates leading to cooperativity and formation of oriented sheets of aromatic ring-systems as reflected in the optical activity induced in the metachromatic band of the achiral drug. No base-pair specificity could be confirmed; however, a high affinity of AMSA to poly(A) chains was demonstrated. The CD measurements did not indicate any significant interaction with RNA. The selectivity of the AMSA-DNA interaction can be regarded as an important argument in favor of the role of this interaction in the antitumor effect of the drug. (Author abstract) (20 Refs)

25. THE STRUCTURE OF CC-1065, A POTENT ANTITUMOR AGENT, AND ITS BINDING TO DNA.

Chidester CG, Krueger WC, Mizsak SA, Duchamp DJ, Martin DG
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J Am Chem Soc; 103(25):7629-7635 1981

The crystal and molecular structure of a potent antitumor agent, CC-1065 (C37H33N7O8), has been determined and circular dichroism studies have demonstrated a strong interaction between CC-1065 and DNA. The triclinic unit cell, space group P1, of dimensions a = 11.063 A, b = 13.311 A, c = 13.405 A, alpha = 85.18 (1), beta = 99.52, and gamma = 103.36, contains two CC-1065 molecules and disordered solvent molecules (2 methanols and 3 waters). Intensity data for 6284 reflections were collected at-155 C; the final R value was 0.08 for 2,411 significant high-angle reflections. NMR spectroscopy was very important in assigning kinds of atoms. The CC-1065 molecule consists of three substituted benzodipyrrole moieties linked by amide bonds. One terminal moiety has a cyclopropyl ring; the only asymmetric carbons in the molecule are at the cyclopropyl ring junctures. The two symmetry-independent molecules have the same configuration, but the absolute configuration is not known. The CC-1065 molecules are curved and somewhat twisted with the outer periphery hydrophilic and the inner surface lipophilic. The interaction between CC-1065 and a number of DNA polymers was studied by circular dichroism. The CD effect requires double-stranded DNA and is strongest with poly(dA-dT):poly(dA-dT). (Author abstract) (28 Refs)

26. A COMPARISON OF THE CARCINOGEN-DNA ADDUCTS FORMED IN RAT LIVER IN VIVO AFTER ADMINISTRATION OF SINGLE OR MULTIPLE DOSES OF N-METHYL-4-AMINOAZOBENZENE.

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Chem Biol Interact; 38(1):15-27 1981

The compound N-methyl-4-aminoazobenzene (MAB) is believed to be metabolized in the liver to an electrophilic N-sulfonyloxy ester which binds covalently to cellular macromolecules, resulting in the induction of hepatic neoplasia. A single dose of MAB is not sufficient for complete carcinogenic activity, so we sought to identify the MAB-DNA adducts present in rat liver after multiple oral doses of [3H]MAB. The MAB was administered by intubation at a level of 0.2 mmol/kg for 1, 3 or 4 doses and animals were sacrificed at 8 hr after the last dose. Hepatic DNA was isolated by extraction and hydroxylapatite chromatography and was enzymatically hydrolyzed to MAB-mononucleoside adducts, which were quantitated by high pressure liquid chromatography (HPLC). After three doses, N2-dG, C8-dG, and an unknown adduct were detected. By four doses, these accounted for 51%, 25% and 23% of the total adducts. This data is consistent with rapid removal of the C8-dG derivative and the relative persistence of the N2-dG and the unknown adduct. The latter was shown to exhibit chromatographic and pH-dependent solvent partitioning properties that were identical to a product also present in DNA treated with the synthetic ultimate carcinogen, N-benzoyloxy-N-methyl-4-aminoazobenzene. Analysis of this adduct by field desorption mass spectrometry and, after perdeuteromethylation, by electron mass spectrometry, indicated the structure to be a deoxyadenosin-N6-yl derivative substituted through an aromatic ring of MAB. Further analysis of 270 MHz 1H-NMR spectroscopy allowed complete assignment of the MAB and adenyl resonances and was uniquely consistent with a 3-(deoxyadenosin-N6-yl)-MAB structure. Since this persistent adduct is potentially mutagenic due to possible tautomeric equilibria between the N6-amino and N6-imino structures, it may represent an initiating lesion in MAB hepatocarcinogenesis. (26 Refs)

27. THE NUCLEOTIDE SEQUENCES OF REARRANGED AND GERMLINE IMMUNOGLOBULIN VH GENES OF A MOUSE MYELOMA MC101 AND EVOLUTION OF VH GENES IN MOUSE.

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J Biol Chem; 257(1):277-285 1982

A rearranged variable region (VH) gene of the immunoglobulin heavy chain was isolated as a part of the complete gamma1 chain gene cloned from a phage library containing DNA derived from a mouse myeloma, MC101. A germline counterpart of the VH gene (VH101) was also obtained from a phage library of mouse fetal liver DNA. The complete nucleotide sequences of both rearranged and germline VH101 genes were determined; these sequences indicate that the rearranged VH101 gene comprises three separate germline DNA segments, the germline VH101 gene, a D (diversity) segment, and the J3 segment. The putative transcription initiation site was estimated to be 30 bases 5' to the initiator codon ATG by the determination of the 5'-terminus of the MC101 mRNA. The nucleotide sequences (260 bp) surrounding the transcription initiation site were found to be the same in the germline and rearranged VH101 genes. The nucleotide sequence of the VH101 gene was compared with those of four other VH genes taken from the literature and the evolutionary relationships of these VH genes were examined using the synonymous nucleotide substitutions as a molecular clock. The branching points of the VH genes were estimated to range from 4 to more than 80 million yr ago in the phylogenetic tree. The results indicate that some of the VH gene ancestors evolved in remote time, probably before the diversification of mammalian species, whereas some of the VH genes diverged much more recently. The latter possibility was supported in part by considerable difference in the sizes and numbers of restriction DNA fragments hybridized to the VH101 probe among DNAs of various subspecies of strains of the genus Mus. (Author abstract) (52 Refs)

28. ANTIBODY DIVERSITY: SOMATIC HYPERMUTATION OF REARRANGED VH GENES.

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Cell; 27(3):573-581 1981

The immune response to phosphorylcholine in BALB/c mice has been well characterized. Amino acid sequence analyses of heavy-chain variable (VH) regions from 19 myeloma and hybridoma immunoglobulins binding phosphorylcholine show that 10 are identical (the prototype T15 VH sequence) and 9 are distinct variants differing by one to eight residues. A T15 VH DNA probe was used to isolate four closely related members of the T15 VH gene family, including one encoding the T15 VH sequence, from a sperm genomic library. A comparison of the protein and germline VH sequences suggested that most of the immune response to phosphorylcholine is derived from the T15 germline VH gene segment. The variant heavy chains from the M167 and M603 alpha immunoglobulins differ in their VH protein sequences from T15 by eight and three residues, respectively. We analyzed the somatic variability in and around the coding regions of these two variant VH genes by comparing them with the corresponding regions of the appropriate germline gene segments. The somatic variation has three properties: it is extensive and is found in flanking as well as coding sequences (for example, at least 44 substitutions for the M167 sequence and 10 substitutions for the M603 sequence); in the coding regions, it includes many silent as well as replacement substitutions; and it is focal in nature and centered around the rearranged VH genes. Although the mutations extend into the neighboring upstream and downstream flanking sequences, sequences approx 5 kb upstream and downstream from the VH genes show no substitutions. Moreover, the associated heavy-chain constant genes (Calpha) from both variant alpha genes are unaltered, indicating that a closely linked and coexpressed gene is unmutated. W e conclude that this somatic variation is generated by a special hypermutational mechanism highly localized in its site of execution and highly restricted in its time of operation during B-cell development. (Author abstract) (43 Refs)

29. SOMATIC DIVERSIFICATION IS REQUIRED TO GENERATE THE VKAPPA GENES OF MOPC 511 AND MOPC 167 MYELOMA PROTEINS.

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Proc Natl Acad Sci USA; 78(12):7674-7678 1981

The immune response to phosphocholine in BALB/c involves one group of heavy chain variable region (VH) genes and at least three groups of light chain variable region (Vkappa) genes, represented by the gene products of the mye-

lomas TEPC 15, MOPC 603, and MOPC 167/MOPC 511. The amino acid sequences of BALB/c myeloma kappa chains MOPC 167 and MOPC 511 are known, and they differ by six amino acids. We have isolated several closely related V region genes of immunoglobulin light chains from a mouse sperm DNA phase library, selecting clones that cross-hybridize with a cDNA plasmid probe encoding the light chains of MOPC 167. We identified six strongly hybridizing clones, representing three separate cloning events. We determined the sequence of the coding and immediate flanking regions of three clones, representing the three separate cloning events, and they proved to be identical. This germ-line sequence encoded the amino acid sequence of neither MOPC 167 nor MOPC 511, but required four base pair changes to generate the VkappaM167 cDNA sequence and five base pair changes to generate the VkappaM511 gene. By Southern hybridization experiments, we demonstrated that neither MOPC 511 nor MOPC 167 germline genes exist. We conclude that the VkappaM167 and VkappaM511 genes are created somatically. (Author abstract) (35 Refs)

30. STRUCTURE OF THE HUMAN IMMUNOGLOBULIN MU LOCUS: CHARACTERIZATION OF EMBRYONIC AND REARRANGED J AND D GENES.

Ravetch JV, Siebenlist U, Korsmeyer S, Waldmann T, Leder P
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Cell; 27(3):583-591 1981

The variable portion of an immunoglobulin heavy chain gene is assembled from at least three discontinuous segments of DNA, the V, D and J regions. We have characterized a 30 kb segment of the human genome that plays a critical role in the formation of this variable region as well as in the expression of the two heavy chains that appear earlier in immunocyte ontogeny, the mu and delta chains. This region encodes nine J-like segments, an interspersed D segment, recombination signals and the genes responsible for the production of both mu and delta heavy chains. Six of the nine germline J-like genes appear to be active and easily account for most of the known human heavy chain amino acid sequences. Three of the J-lik e genes are pseudogenes. The large number of human J region genes and, hence, their greater potential for generating diversity as compared to that of the mouse J region genes appears to result from recent genetic duplications. To assess this potential, we have analyzed two recombinant genes involving this region. One of these, a V-D-J recombinant, is likely to be an active gene; the other, an aberrant D-J recombinant. A comparison of the structures of these D regions raises the possibility that they are mosaics formed by recombination between D segments. (Author abstract) (37 Refs)

31. ORGANIZATION AND REORGANIZATION OF IMMUNOGLOBULIN GENES IN A-MULV-TRANSFORMED CELLS: REARRANGEMENT OF HEAVY BUT NOT LIGHT CHAIN GENES.

Alt F, Rosenberg N, Lewis S, Thomas E, Baltimore D
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Cell; 27(2):381-390 1981

The structure of immunoglobulin-related genes was analyzed in individual Abelson murine leukemia virus (A-MuLV)-transformed lymphoid cell lines. Essentially all of these lines, whether immunoglobulin-containing or null, had DNA rearrangements in the vicinity of the JH regions on both chromosomes as well as deletions of at least 5 kb of DNA 5' to JH. None of these lines, however, except rare light chain producers had detectable rearrangements at either their kappa or lambda light chain loci. In contrast to A-MuLV-transformants derived from bone marrow, those from early fetal liver frequently contained more than 2 and sometimes 12 or more distinct, rearranged JH-containing fragments. Cellular subclones derived from these lines had a subset, usually two, of the fragments found in the parent line. Therefore, heavy chain gene rearrangement appears to precede that of light chain gene rearrangement and is still continuing in certain cultured A-MuLV transformants. (Author abstract) (43 Refs)

32. GENE AMPLIFICATION IN METHOTREXATE-RESISTANT MOUSE CELLS. II. REARRANGEMENT AND AMPLIFICATION OF NON-DIHYDROFOLATE REDUCTASE GENE SEQUENCES ACCOMPANY CHROMOSOMAL CHANGES.

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Medical Res. Council Mammalian Genome Unit, King's Building, West Mains Road, Edinburgh EH9 3JT, Scotland
J Mol Biol; 153(2):219-236 1981

Total amplified DNA in methotrexate-resistant mouse lymphoma EL4 cells and mouse melanoma PG19 cells has been characterized in two ways. Metaphase spreads show the presence of additional chromosome forms that are either 'homogeneously staining' chromosomes or 'double minute' and ring chromosomes. Gel electrophoresis of restriction enzyme-digested nuclear DNA shows the presence of amplified sequences, the pattern of which is unique in each of five cell lines. We conclude that extensive DNA rearrangement has taken place during amplification. (Author abstract) (38 Refs)

33. GENE AMPLIFICATION IN METHOTREXATE-RESISTANT MOUSE CELLS. III. INTERRELATIONSHIPS BETWEEN CHROMOSOME CHANGES AND DNA SEQUENCE AMPLIFICATION OR LOSS.

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J Mol Biol; 153(2):237-256 1981

The amplified DNA sequences within a single subline of methotrexate-resistant mouse cells have a complex intracellular location, some being associated with 'double minute' chromosomes, and some with normal-sized chromosomes. However, within a cell line and probably within a cell their internal structure is stable and homogeneous. These results, and those from the previous paper, suggest that gene amplification in these cells has two distinct stages. Firstly, an amplifiable unit is generated by a process involving DNA rearrangement. Secondly, the unit increases in number but remains unchanged in structure. (Author abstract) (17 Refs)

34. RELATIONSHIP OF AMPLIFIED DIHYDROFOLATE REDUCTASE GENES TO DOUBLE MINUTE CHROMOSOMES IN UNSTABLY RESISTANT MOUSE FIBROBLAST CELL LINES.

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Mol Cell Biol; 1(12):1077-1083 1981

The localization of unstably amplified dihydrofolate reductase (DHFR) genes to double minute (DM) chromosomes was determined in the methotrexate (MTX) resistant 3T6 and 3T3 mouse cell lines. Cells were labeled with a fluoresceinated de-

rivative of MTX and analyzed by a fluorescence-activated cell sorting technique. 3T6 cells selected in increasing concentrations of MTX were unstable for DHFR overproduction and MTX resistance when cultured in the absence of MTX. Karyotypic analyses of both cell lines revealed DM chromosomes not present in the parental cell strains. Essentially identical kinetics of DHFR gene sequence loss of total DNA and of DNA from fractions enriched in DM chromosomes were observed during reversion to MTX sensitivity; the finding suggested localization of unstably amplified gene sequences on DM chromosomes. Analysis of cell populations sorted according to DHFR content showed a proportional relationship between gene amplification and enzyme content in the DM chromosome. (27 Refs)

OTHER RELATED STUDIES

35. IMPROVEMENTS TO A PROGRAM FOR DNA ANALYSIS: A PROCEDURE TO FIND HOMOLOGIES AMONG MANY SEQUENCES.

Queen C, Wegman MN, Korn LJ
Center Cancer Res., Massachusetts Inst. Technology, Cambridge, MA, 02139
Nucleic Acids Res; 10(1):449-456 1982

We have devised an algorithm for finding partial homologies among a set of nucleotide sequences. The algorithm and other improvements have been incorporated into a commonly used computer program for the analysis of sequence data. (Author abstract) (36 Refs)

36. DNA CHARACTERISTICS OF HUMAN PITUITARY TUMOURS.

Anniko M, Holm LE, Tribukait B, Werner S
Dept. Otolaryngology, Karolinska Hosp., S-104 01 Stockholm, Sweden
Acta Otolaryngol [Suppl] (Stockh); (379):5-11 1981

Biopsies from pituitary tumors in eight patients were analyzed with DNA flow-cytofluorometry immediately after surgery and after organ culture. Good agreement was found between the degrees of ploidy and of the relative proportions of cells in different phases of the cell cycle, indicating a good stability and reproducibility of pituitary tumor explants in vitro. Analysis of DNA content indicated a homogeneous cell population in tumors before and after explanation. No apparent correlation was observed between hormone secretion rate in diploid and aneuploid tumors, respectively, and DNA characteristics. (Author abstract) (19 Refs)

37. CELLULAR DNA CONTENT OF PITUITARY TUMOURS: ITS RELATIONSHIP TO MORPHOLOGY AND HORMONAL TYPE OF TUMOUR.

Anniko M, Holm LE, Silfversward C, Tribukait B, Wersall J
Dept. Otolaryngology, Karolinska Hosp., S-104 01 Stockholm, Sweden
Acta Otolaryngol [Suppl] (Stockh); (379):13-19 1981

The DNA content of 24 human pituitary tumors was analyzed with flow-cytofluorometry, and compared with the morphologic characteristics of the tumors. Fifteen tumors had a diploid and 9 had an aneuploid DNA pattern. Minimal and moderate degrees of mitosis and nuclear polymorphism can possibly occur more frequently in aneuploid pituitary tumors than in diploid pituitary tumors (mitoses: 56% vs 27%; nuclear polymorphism: 78% vs 53%). Otherwise no correlation between the degree of ploidy and the histopathologic or ultrastructural features could be registered. Thus, pituitary tumors of the same morphology can be subdivided according to their ploidy pattern. (Author abstract) (17 Refs)

38. THE CLINICAL IMPLICATIONS OF DNA CHARACTERISTICS IN HUMAN PITUITARY TUMOUR DISEASE.

Anniko M, Holm LE, Tribukait B, Werner S, Wersall J
Dept. Otolaryngology, Karolinska Hosp., S-104 01 Stockholm, Sweden
Acta Otolaryngol [Suppl] (Stockh); (379):21-28 1981

DNA analysis was performed in 29 cases of various endocrinological types of human pituitary tumors. The degrees of ploidy and proliferation, expressed as the proportion of cells in S phase, were determined. In 12 cases (41%) aneuploidy occurred. The proportion of aneuploid cells in these tumors was very high, in general around 95% measured. The aneuploid group of pituitary tumors showed a large variation of the percentage of cells in S-phase (range: 3.3-13.7%; mean: 8.3 +-5.3) in contrast to diploid tumors which showed a considerably smaller variation (range: 3.4-8.8%; mean 5.5 +-2.1). Patients with aneuploid tumors had a mean age at operation of 42.8 +-11.1 yr in contrast to 51.5 +-13.8 yr for patients wi th diploid tumors. Aneuploidy occurred more frequently in tumors causing acromegaly with a concomitant secretion of GH and PRL than in other endocrinological types of tumors. Patients with aneuploid tumors had a mean case history of 2.9 +-1.9 yr, as compared to 10.5 +-4.9 yr for patients with diploid tumors. In many cases, tumors with an aneuploid DNA pattern caused a high clinical activity of acromegaly. (Author abstract) (28 Refs)

39. CLINICOPATHOLOGICAL STUDIES OF DNA CONTENT IN NUCLEI OF CELLS ISOLATED FROM PATIENTS WITH ESOPHAGEAL CARCINOMA.

Hiratsuka R
Second Dept. Surgery, Sch. Medicine, Fukuoka Univ., Fukuoka 812, Japan
Fukuoka Igaku Zasshi; 72(10):556-574 1981

Nuclear DNA content in cells from 37 patients with esophageal cancer was measured by means of microfluorometry and Feulgen staining. Patients were divided into four groups according to DNA histogram patterns: Type I (7 patients) had cells that were generally hypotriploid, with less than 30% hypertetraploid. Type II (7 patients) had cells between diploid and tetraploid, with greater than 30% hypertetraploid. Type III (12 patients) had cells in the tetraploid-hexaploid range, with less than 60% greater than tetraploid. Type IV (12 patients) had cells in the tetraploid-hexaploid range, with greater than 60% greater than tetraploid. Poorly differentiated squamous cell carcinoma occurred in 5/12 Type IV patients but not in any Type I patients. Lymphatic permeation occurred in 1/7 Type I and 7/12 Type IV patients. Marked lymphatic metastasis occurred in 4/7 Type I and 11/12 Type IV patients. Survival time was greater than 3 yr for 4/5 Type I patients but less than 1 yr for 7/11 Type IV patients, who died of recurrent cancer. Biopsy specimens from 15/26 recurrent tumors had the same DNA histogram patterns as the original tumors. Because unusual characteristics make morphological staging alone inadequate for prognosis of gastric cancer, evaluation of DNA content is recommended in conjunction with staging. (39 Refs) (In JPN)

* U.S. GOVERNMENT PRINTING OFFICE: 1982—361-847/119

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MECHANISMS OF DNA REPLICATION

1. DNA CHAIN ELONGATION RATES IN MARROW CELLS FROM VITAMIN B12-DEFICIENT PATIENTS AND METHOTREXATE-TREATED MICE.

Bond AN, Harris G, Wickramasinghe SN
(c/o Wickramasinghe) Dept. Hematology, St. Mary's Hosp. Medical Sch., London W2 1PG, England
Br J Haematol; 50(2):299-307 1982

The DNA synthesized by marrow cells from patients with vitamin B12 deficiency and mice previously given methotrexate (MTX), has been investigated. Suspensions of bone marrow cells were pulse-labeled with [methyl-3H]thymidine or deoxy[5-3H]cytidine for 30 seconds and the radioactivity in the

HE 20.3123/2:

DNA was chased thereafter in the presence of 10 uM non-radioactive nucleoside for periods up to 60 min. The rates of elongation of new daughter strands were then assessed by hydroxyapatite chromatography of alkali-denatured DNA samples. No significant differences were found between the av rates of elongation of daughter strands from control marrow cells on the one hand and the vitamin B12-deficient or the methotrexate-affected cells on the other. This is to be contrasted with the results of previous studies which have shown a retardation in the rates of movement of replication forks in stimulated, cultured lymphocytes obtained from vitamin B12-or folate-deficient patients. (Author abstract) (17 Refs)

2. X-RAY INHIBITION OF DNA SYNTHESIS AT DISCRETE TIMES DURING S PHASE IN SYNCHRONOUS HUMAN DIPLOID FIBROBLASTS.

Kapp LN, Painter RB
Lab. Radiobiology, Univ. California, San Francisco, CA, 94143
Radiat Res; 89(2):424-427 1982

The incorporation of thymidine into irradiated and unirradiated diploid fibroblasts at various times during S phase was investigated. Results indicated that most replicons initiate during relatively restricted times in S phase. Results support a model of diploid fibroblast S phase that consists of multiple initiations of large numbers of replicons during short, specific times. (12 Refs)

EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

3. THE EFFECTS OF INHIBITORS OF DNA BIOSYNTHESIS ON THE CYTOTOXICITY OF 6-THIOGUANINE.

Lee SH, Sartorelli AC
Comprehensive Cancer Center, Yale Univ. Sch. Medicine, New Haven, CT, 06510
Cancer Biochem Biophys; 5(3):189-194 1981

The effects of hydroxyurea, 1-beta-D-arabinofuranosylcytosine, and 5-fluorodeoxyuridine on the antiproliferative activity of 6-thioguanine (6-TG) in exponentially growing L1210 leukemia cell cultures were measured by changes in (methyl-3H)thymidine, (U-3H)uridine, and (8-14C)adenine incorporation. None of the inhibitors of DNA biosynthesis caused additive toxicity when used with 6-TG but instead significantly decreased the delayed cytotoxicity of 6-TG, suggesting that cell injury caused by the agent is mediated at least in part through DNA synthetic processes. Cells exposed to 10 uM 6-TG for 30 min in G1/S or S phases of the cell cycle were considerably more susceptible to the cytotoxic action of 6-TG than those treated in G2 phase. However, some cytotoxicity was also evident after exposure in G2 phase, suggesting that multiple biochemical lesions account for the effects. Alkaline sucrose gradient sedimentation of L1210 cells exposed to 6-TG revealed that the purine analogue causes structural breaks in DNA. (35 Refs)

4. A LONG-LIVED THYMIDINE POOL IN EPITHELIAL STEM CELLS.

Hume WJ, Potten CS
Paterson Lab., Christie Hosp. and Holt Radium Inst., Manchester M20 9BX, England
Cell Tissue Kinet; 15(1):49-58 1982

The labelling index (LI) of the individual basal cell positions of the anterior column of mouse tongue filiform papillae was assessed with time after an injection of [3H]TdR at 12.00 hr (the minimum point in the circadian LI rhythm). An initial doubling of the LI in the stem cell zone due to cell division was followed by a second rise of 14-16% 16 hr after injection and this occurred even in the presence of vincristine. Although the uptake of [3H]TdR and the initial LI doubling were largely prevented by a preceding injection of hydroxyurea, the 14-16% LI rise was still observed. The possible explanations are discussed, the favored one being that an av of one of the six or seven cells (the stem cell) in each stem cell zone can store [3H]TdR in a long-lived precursor pool for at least 16 hr before being utilized for DNA synthesis. This complements previously published work which suggested that one cell in each stem cell zone may selectively segregate DNA at mitosis. (Author abstract) (45 Refs)

5. CYTOTOXICITY, MUTAGENICITY AND DNA DAMAGE BY HOECHST 33342.

Durand RE, Olive PL
Section of Radiobiology, Johns Hopkins Oncology Center, Baltimore, MD, 21205
J Histochem Cytochem; 30(2):111-116 1982

Hoechst 33342 can be used for flow microfluorimetry (FMF) analysis of the DNA content of living Chinese hamster cells, giving good resolution (coefficients of variation (CVs) approx 6%) with relatively nontoxic staining regimens. The dye is, however, a very efficient inhibitor of DNA synthesis, with a marked depression of the DNA synthetic rate for V79 cells observed at concentrations 10-fold less than those required for optimal FMF resolution. Nontoxic and minimally toxic Hoechst concentrations also resulted in demonstrable mutation, as assayed by 6-thioguanine resistance. Hoechst disappearance from cells returned to normal growth medium after staining suggests two components of binding, since about half of the total strain is rapidly removed, whereas the rest is apparently diluted only by cell division. Cells containing Hoechst 33342 die more rapidly than control cells when held at 4 C and are also more susceptible to inactivation by the ultraviolet laser beam if operated at approx greater than or equal to 100 mW power. Thus, Hoechst 33342 can provide information about the DNA content of living cells, but at the expense of minimal toxicity, moderate mutation, and significant cell cycle perturbations. (Author abstract) (12 Refs)

6. ANTITUMOR AGENTS XLVII: THE EFFECTS OF BISBRUSATOLYL MALONATE ON P-388 LYMPHOCYTIC LEUKEMIA CELL METABOLISM.

Hall IH, Liou YF, Lee KH, Okano M, Chaney SG
Div. Medicinal Chemistry, Sch. Pharmacy, Univ. North Carolina at Chapel Hill, Chapel Hill, NC, 27514
J Pharm Sci; 71(2):257-262 1982

Bisbrusatolyl malonate, which was shown previously to be active against P-388 lymphocytic leukemia cell growth, was investigated for inhibitory effects on nucleic acid and protein synt hesis. DNA and RNA synthesis as well as protein synthesis were markedly inhibited at 10, 25 and 50 umole final con-

centrations in vitro. The major sites of inhibition of nucleic acid synthesis appeared to be DNA polymerase, messenger RNA polymerase and transfer RNA polymerase, orotidine-5'-monophosphate decarboxylase, phosphoribosyl pyrophosphate amino transferase, and dihydrofolate reductase. Moderate inhibition of nucleotide kinase activities and oxidative phosphorylation processes occurred after drug treatment. Cyclic adenosine monophosphate levels were reduced. Protein synthesis was inhibited during the elongation step of peptide synthesis. The data suggested that bisbrusatolyl malonate interfered with the peptide bond formation. However, the ongoing polypeptide synthesis must be completed before the drug can bind to the ribosome effectively. (Author abstract) (41 Refs)

7. COMPARATIVE STUDIES ON ANTI-TUMOR EFFECTS OF NITROSOUREAS, ACNU AND MCNU, ON EXPERIMENTAL BRAIN TUMORS.

Harada K, Kiya K, Okamoto H, Uozumi T
Dept. Neurosurgery, Hiroshima Univ., Hiroshima 734, Japan
Neurol Med Chir (Tokyo); 21(10):1017-1023 1981

Comparative anti-tumor effects of ACNU (1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2 chloroethyl)-3 nitrosourea) and MCNU were studied on 20-methylcholanthrene-induced C57 black mouse malignant gliomas (MG-cells) and rat glial tumors (C6-cells) in vitro and in vivo. The inhibitory effects of ACNU and MCNU in vitro were dependent on dose and time. The concentration showing inhibitory effect was more than 10 ug/ml. ACNU was slightly more effective than MCNU. ACNU significantly inhibited DNA synthesis at concentrations higher than 10 ug/ml, but had no such effect on RNA and protein syntheses. MCNU also inhibited DNA synthesis similar to but less than that of ACNU. By a single intraperitoneal administration on day 5, an increased life span (ILS) was observed with ACNU (120.3% increase) and with MCNU (100.4%). The optimal dose was 25 mg/kg/day for both drugs. The therapeutic ratio, or optimal dose divided by the dose producing an increased life span of 30% over controls, of ACNU was 4.17 and that of MCNU was 6.67. When 25 mg/kg/day of ACNU was administered intraperitoneally on day 5, 2 out of 10 mice survived over 60 days. In case of MCNU, 1 out of 10 mice survived. A single administration of ACNU or MCNU was more effective for the increased life span compared with intermittent administration. From these results, it is suggested that the new water soluble nitrosourea, MCNU, can be utilized as a chemotherapeutic agent against brain tumors as in the case of ACNU. (Author abstract) (28 Refs) (In JPN)

8. NEOPLASTIC TRANSFORMATION OF SYRIAN HAMSTER EMBRYO CELLS BY BISULFITE IS ACCOMPANIED WITH A DECREASE IN THE NUMBER OF FUNCTIONING REPLICONS.

Doniger J, O'Neill R, DiPaolo JA
Lab. Biology, NCI, Bethesda, MD, 20205
Carcinogenesis; 3(1):27-32 1982

Bisulfite, a chemical that at neutral pH does not induce mutations at two loci in Chinese hamster V-79 cells, induces transformation of Syrian hamster embryo cells. Although bisulfite affects DNA metabolism, there was no evidence of bisulfite-induced DNA damage. Bisulfite induced no excision repair replication, caused no DNA strand breaks detectable in alkaline sucrose gradients, had no effect on the size distribution of DNA nascent daughter strands, and did not affect excision on post-replication repair of uv-induced damage. However, bisulfite did induce a dose-dependent decrease in the rate of DNA replication per cell, apparently due to a reduction in the number of functioning replicons. Since the data indicate that bisulfite causes no detectable DNA damage, it appears that bisulfite induces transformation by a non-mutagenic event that could involve the inhibition of semi-conservative DNA synthesis. (Author abstract) (27 Refs)

9. PARTIAL PURIFICATION OF A LOW MOLECULAR WEIGHT INHIBITOR OF EPIDERMAL DNA SYNTHESIS.

Quirk AV, Pain RH, Davison M, Shuster S
(c/o Shuster) Dept. Dermatology, The University, Newcastle upon Tyne NE1 7RU, England
Cell Tissue Kinet; 15(1):31-38 1982

An attempt has been made to purify factors present in aqueous extracts of pig epidermis which inhibit epidermal cell proliferation. A lipophilic factor of low molecular wt (less than 10,000), has been shown to inhibit DNA synthesis as measured by the uptake of tritiated thymidine in mouse ear epidermis. Purification by alcohol precipitation, ethyl acetate extraction and silicic acid column chromatography produced a 15-fold increase in the specific activity of the inhibitory action. It seems likely that aggregation or absorption of this low molecular wt factor may explain the high molecular wt of epidermal cell proliferation inhibitors previously studied, as well as the difficulty in their characterization. (Author abstract) (12 Refs)

10. ESTROGEN REGULATION OF GROWTH AND SPECIFIC PROTEIN SYNTHESIS IN HUMAN BREAST CANCER CELLS IN TISSUE CULTURE.

Edwards DP, Adams DJ, McGuire WL
Dept. Medicine, Univ. Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX, 78284
Adv Exp Med Biol; 138:133-149 1982

New protein markers of estrogen action and dependence of progesterone receptors upon estrogen-stimulated cell growth were studied using MCF-7 human breast cancer cells in tissue culture. Results showed that estradiol stimulates DNA polymerase activity in normal target tissues. Progesterone receptor induction and estrogen-mediated cell division may involve separate control mechanisms. A specific 24,000 mol wt protein may be a marker of estrogen-stimulated growth of human breast tumor cells. (27 Refs)

11. HEART MITOCHONDRIAL DNA SYNTHESIS: PREFERENTIAL INHIBITION BY ADRIAMYCIN.

Hixon SC, Ellis CN, Daugherty JP
Dept. Biochemistry, Comprehensive Cancer Center, Univ. Alabama, Univ. Station Birmingham, Birmingham, AL, 35294
J Mol Cell Cardiol; 13(9):855-860 1981

The effect of adriamycin (ADR) on mitochondrial (mi) and nuclear (nu) DNA synthesis was determined in the heart and liver of female Sprague-Dawley rats. In an in vitro study, ADR was added to appropriate suspensions at concentrations of 2 or 5 ug/ml. DNA synthesis was determined by (3H)thymidine incorporation during 50 min of incubation. Compared to control preparations, ADR inhibited both heart and liver miDNA synthesis; nuDNA synthesis was also inhibited but the response was not as dose-dependent as with mitochondria. Rate of DNA synthesis in heart nuclei was low compared to that in liver nuclei, reflecting the absence of ongoing mitosis in myocytes. In an in vivo study, after a single dose of 10 mg/kg of ADR/rat, DNA synthesis was studied subsequently on days 2, 5, 7, and 15. Each rat was killed precisely 30 min after injection of 1 mCi/kg of (3H)thymidine. Both

heart and liver nuDNA synthesis were markedly inhibited on day 2 and 4 with rebound by day 7 in liver and day 10 in heart. Heart and liver miDNA was not as inhibited as nuDNA. The in vivo inhibition of DNA could be influenced by a general toxic effect of ADR as reflected by losses in wt of treated rats. The significance of these results in relation to ADR cardiotoxicity is discussed. (15 Refs)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

12. DNA-POLYMERASE INDUCED BY HERPESVIRUS PAPIO (HVP) IN CELLS OF LYMPHOBLASTOID CULTURES DERIVED FROM LYMPHOMATOUS BABOONS. - REPORT V.

Djachenko AG, Lapin BA

(c/o Lapin) Inst Experimental Pathology and Therapy, USSR Acad. Medical Sciences, Gora Trapetziya, P. B. 66, Sukhumi, USSR

Exp Pathol, 20(1):51-57 1981

A new DNA-polymerase was found in the cells of suspension lymphoblastoid cultures which produce lymphotropic baboon herpesvirus (HVP). This enzyme was isolated in a partially purified form. Some of its properties vary from those of other cellular DNA-polymerases. HVP-induced DNA-polymerase has a molecular weight of 160,000 and sedimentation coefficent of about 8 S. The enzyme is resistant to high salt concentration and N-ethylmaleimide, but it is very sensitive to phosphonoacetate. It effectively copies 'activated' DNA and synthetic deoxyribohomopolymers. Attempts to reveal the DNA-polymerase activity in HVP virions were unsuccessful. (Author abstract) (15 Refs)

13. DNA METHYLASE ACTIVITY OF NORMAL LIVER, REGENERATING LIVER, AND A TRANSPLANTABLE HEPATOCELLULAR CARCINOMA.

Walker MS, Becker FF

(c/o Becker) Dept. Pathology, Univ. Texas System Cancer Center, M.D. Anderson Ho sp. and Tumor Inst., Houston, TX, 77030

Cancer Biochem Biophys; 5(3):169-173 1981

DNA methylase was obtained from regenerating rat liver (RRL) from male ACI animals in which transplantable hepatocellular carcinoma (THC 252) had been induced by exposure to N-2-acetylaminofluorene, from normal rat livers (NRL), and from THC 252 nuclei (THC). The activities of DNA methylase in crude extracts of NRL, RRL, and THC, determined by methylation of denatured Micrococcus luteus DNA, were as follows: NRL less than RRL less than THC. A similar assay with Escherichia coli and calf thymus DNAs, both single- and double-stranded, confirmed that methylase activities of the three tissues remain in the same order regardless of substrate. Methylation saturation studies performed with DNAs from the same three rat tissues showed that THC 252 DNA is hypomethylated, and that the undermethylation does not result from an inability of THC 252 methylases to methylate THC 252 DNA. The results confirm earlier findings obtained by direct analysis of 5-methylcytosine content; chromatin composition or configuration is the most likely explanation for hypomethylation. (25 Refs)

14. INHIBITION OF THYMIDYLATE SYNTHASE BY HYDROXYUREA IN RAPIDLY PROLIFERATING P815 MASTOCYTOMA CELLS.

Boehm TL, Kreis W, Drahovsky D

Zentrum der Biologischen Chemie, Universitat Frankfurt am Main, D-6000 Frankfurt/Main, W. Germany

Biochim Biophys Acta; 696(1):52-56 1982

The incorporation of [14C]deoxycytidine, [3H]deoxyuridine, and [3H]thymidine, respectively into pyrimidine bases of DNA has been measured in rapidly proliferating P815 mouse mastocytoma cells in the presence of hydroxyurea. The incorporation of [14C]deoxycytidine-derived radioactivity into DNA cytosines is increased when compared to the incorporation into DNA thymines. The [3H]deoxyuridine-derived radioactivity is incorporated solely into DNA thymines and this incorporation is inhibited by hydroxyurea in a dose-dependent manner. This suggests an inhibitory effect of hydroxyurea on the thymidylate synthase which was proved in experiments in which the conversion of deoxyuridine monophosphate into deoxythymidine monophosphate catalyzed by a crude enzyme preparation from P815 cells was inhibited in the presence of hydroxyurea. Enzymatic DNA methylation as measured by the conversion of incorporated [14C]deoxycytidine into 5-methylcytosines was not affected by hydroxyurea. (Author abstract) (10 Refs)

MECHANISMS OF DNA REPAIR

15. REJOINING OF DNA DOUBLE-STRAND BREAKS IN HUMAN FIBROBLASTS AND ITS IMPAIRMENT IN ONE ATAXIA TELANGIECTASIA AND TWO FANCONI STRAINS.

Coquerelle TM, Weibezahn KF

Kernforschungszentrum Karlsruhe, Institut fur Genetik und fur Toxikologie von Spaltstoffen, Postfach 3640, D-7500 Karlsruhe 1, W. Germany

J Supramol Struct Cell Biochem; 17(4):369-376 1981

Repair of DNA double-strand breaks (DSB) in fibroblasts from normal individuals and patients with ataxia telangiectasia (AT) or Fanconi's anemia (FA) was studied. Cell strains tested included 'Munich' from a normal child, 'Berlin' from a normal adult, AT2BE, AT3BI, and AT5BI from patients with AT, and FA CRL 1196, FA Buse, FA 1424, and FA 3557 from patients with FA. Cell cultures were exposed to gamma-radiation (33 Gray/min for 1.5 min) and incubated at 37 C under aerobic conditions; cells not incubated were irradiated at 0 C. DNA DSB were analyzed by neutral filter elution. The lowest dose of radiation producing measurable DSB was 5 Gray, which produced one DSB/3.2 daltons DNA. At 37 C fibroblasts from normal individuals (adult and child) repaired approx 50% of DSB resulting from 50 Gray within 3 min; after 30 min 90% of DSB were repaired. DSB were repaired by a fast repair mechanism and a slow mechanism. Cells from strain AT2BE showed defective fast and slow repair mechanisms. After long incubation times AT2BE cells retained 4-6% more DSB than normal cells. AT3BI and AT5BI cells showed normal repair mechanisms. FA3557 and FA1424 showed defective fast and slow repair mechanisms. FA CRL 1196 and FA Buse showed normal repair mechanisms. Defective DSB repair is apparently the result of deficient enzyme activity. (20 Refs)

16. DNA REPAIR IN NORMAL HUMAN AND XERODERMA PIGMENTOSUM GROUP A FIBROBLASTS FOLLOWING TREATMENT WITH VARIOUS METHANESULFONATES AND THE DEMONSTRATION OF A LONG-PATCH (U.V.-LIKE) REPAIR COMPONENT.

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Carcinogenesis; 3(1):7-14 1982

Excision repair of DNA in normal and xeroderma pigmentosum complementation Group A fibroblasts was examined following treatment with methyl-methanesulfonate, ethyl-methanesulfonate and isopropyl methanesulfonate. Studies utilizing repair synthesis methods and inhibition with arabinofuranosyl cytosine revealed two distinct phases of repair; one commencing and terminating within the first 3-5 hr after the treatment, and a second, much longer phase extending from 9-35 hr post-treatment. Both phases of repair having a long-patch (uv-like) component, which establishes for the first time the existence of this mode of repair in response to alkane sulfonate damage. While xeroderma cells display somewhat fewer alkaline labile sites in their DNA following alkylation treatment than do their normal counterparts (consistent with earlier observations suggesting a deficiency in a glycosylase or apurinic endonuclease), we are unable to demonstrate a deficiency of these cells in either of the two phases of repair. (Author abstract) (61 Refs)

17. GENETIC ASPECTS OF DNA REPAIR DEFICIENCY.

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Gann Monogr Cancer Res; 27:13-19 1982

DNA repair in human cells, specifically those of persons with xeroderma pigmentosum (XP), is reviewed. A survey of the relative levels of unscheduled DNA synthesis (UDS) in XP patients in Japan revealed a unique XP group, group F, in which patients have mild symptoms despite their apparently low repair capacities. In this group, UDS may not represent the actual capacity for excision repair; the resynthesis step was slower than in normal cells and UDS measured during the first few hr after UV irradiation detected only a small portion of the total repair. Inactivation of colony-forming ability and UDS did not correspond in Bloom's syndrome cells treated with mitomycin C and XP strains did not demonstrate high sensitivity to antipain. The presence of UDS suggests the presence of DNA damage subject to some type of repair, but is not proof. The frequency of the population at genetically high risk of cancer in Japan is reported for XP, ataxia telangiectasia, Bloom's syndrome, Fanconi's anemia, retinoblastoma, familial polyposis coli, and Down's syndrome. Overall, the frequency of the population with a high risk of cancer may be 3%. The genetic factor should not be considered a major cause of cancer in Japan. (16 Refs)

18. EXCISION AND CROSSLINK REPAIR OF DNA AND SISTER CHROMATID EXCHANGES IN CULTURED HUMAN FIBROBLASTS WITH DIFFERENT REPAIR CAPACITIES.

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Gann Monogr Cancer Res; 27:33-44 1982

The relationship between sister chromatid exchange (SCE) induction and DNA repair was studied by evaluating the role of UV excision repair. The roles of interstrand crosslinking and repair in the induction of SCE in fibroblasts of xeroderma pigmentosum (XP) complementation groups (A to G and a variant group), Cockayne's syndrome (CS), and Fanconi's anemia (FA) with different repair capacities and predispositions to malignancies were studied. Killing sensitivities of the three types of cells to UV, 4-nitroquinoline-1-oxide (4NQO), and monofunctional decarbamoyl mitomycin C (DCMC) are reported. The XP groups were deficient in the initial steps of dimer excision repair in the order of A = G greater than C greater than D greater than D = F greater than variant. The extent of dimer-repair deficiency strictly determined the killing sensitivity of XP cells. Studies of the susceptibilities of XP and CS cells to SCEs induced by mutagens demonstrated that in the most defective XP group-A cells, the SCE induction rate by UV, 4NQO, and DCMC was a 3-5-fold higher than in normal cells. FA cells were approx 1.7x more sensitive to SCE induction by MC than normal cells and the SCE rate induced by DCMC was the same as that of normal cells. In XP cells, a six-fold hi gher SCE rate was observed after DCMC, and only a slightly higher SCE rate was observed after MC. Thus, part of cross-link damage may yield SCE. Caffeine synergistically increased UV-induced SCEs. but not UV-induced mutations in V79 cells, thus, dissociated SCE from mutation. (21 Refs)

19. DIFFERING LEVELS OF EXCISION REPAIR IN HUMAN FETAL DERMIS AND BRAIN CELLS.

Gibson RE, D'Ambrosio SM
Dept. Radiology, Ohio State Univ. Comprehensive Cancer Center, Coll. Medicine, Ohio State Univ., Columbus, OH, 43210
Photochem Photobiol; 35(2):181-185 1982

The levels of DNA excision repair, as measured by unscheduled DNA synthesis (UDS) and the UV-endonuclease sensitive site assay, were compared in cells derived from human fetal brain and dermal tissues. The level of UDS induced following ultraviolet (UV) irradiation was found to be lower (approx 60%) in fetal brain cells than in fetal dermal cells. It was determined, using the UV-endonuclease sensitive site assay to confirm the UDS observation, that 50% of the dimers induced by UV in fetal dermal cells were repaired in 8 hr, while only 15% were removed in the fetal brain cells during the same period of time. Even after 24 hr, only 44% of the dimers induced by UV in the fetal brain cells were repaired, while 65% were removed in the dermal cells. These data suggest that cultured human fetal brain cells exhibit lower levels of excision repair compared to cultured human fetal dermal cells. (Author abstract) (31 Refs)

20. CHRONIC OR ACUTE ADMINISTRATION OF VARIOUS DIALKYLNITROSAMINES ENHANCES THE REMOVAL OF O6-METHYLGUANINE FROM RAT LIVER DNA IN VIVO.

Margison GP
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Chem Biol Interact; 38(2):189-201 1982

The effect of pretreatment of rats with various symmetrical dialkylnitrosamines on the repair of O6-methylguanine produced in liver DNA by a low dose of [14C]dimethylnitrosamine (DMN) has been examined. DMN, diethylnitrosamine (DEN), dipropylnitrosamine (DPN) or dibutylnitrosamine (DBN) were administered to rats for 14 consecutive weekdays at a daily dose of 5% of the LD50. Animals were given [14C]DMN 24 hr after the last dose and were killed 6 hr later. DNA was extracted from the liver and ana-

lyzed for methylpurine content after mild acid hydrolysis and Sephadex G-10 chromatography. While the amounts of 3-methyladenine and 7-methylguanine were only slightly different from controls, the amounts of O6-methylguanine in the DNA of the dialkylnitrosamine pretreated rats were about 30% of those in control rats, indicating a considerable increase in the capacity to repair this base. Liver ribosomal RNA from control and dialkylnitrosamine pretreated rats contained closely similar amounts of O6-methylguanine suggesting that the induced enzyme system does not act on this base in ribosomal RNA in vivo. Pretreatment with the se dialkylnitrosamines also enhanced the repair of O6-methylguanine in liver DNA when they were given as a single dose (50% of the LD50) either 3 or 7 days before the [14C]DMN. In addition, single low doses of DMN or DEN (5% of the LD50) given either 1 or 6 days before [14C]DMN increased O6-methylguanine repair and the magnitude of the effect after DEN was similar to that produced by the other pretreatment schedules. The possible mechanism(s) of the induction of O6-methylguanine repair and its relation to hepatotoxicity, DNA alkylation, carcinogenesis and the adaptive response in Escherichia coli are discussed. (Author abstract) (42 Refs)

21. ROLE OF DNA REPAIR IN MUTAGENESIS OF CHINESE HAMSTER OVARY CELLS BY 7-BROMOMETHYLBENZ[A]ANTHRACENE.

Thompson LH, Brookman KW, Carrano AV, Dillehay LE
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Proc Natl Acad Sci USA; 79(2):534-538 1982

The role of DNA repair in mutagenesis was studied in normal, repair-proficient Chinese hamster ovary cells and in two mutant strains that are deficient in excision repair. By using the mutagen 7-bromomethylbenz[a]anthracene (7-BrMeBA) and the technique of alkaline elution of DNA, the mutants were found to be defective at or before the incision step of excision repair. Dose-responses were determined for cell killing, mutation induction at three loci, and sister chromatid exchanges over a survival range of 1.0-0.1 after 7-BrMeBA treatment. The mutants were five-fold more sensitive to killing than were the normal cells, but the degree of hypersensitivity to mutation induction varied depending on the mutant strain, the genetic marker, and the dose of mutagen. In each instance, the dose-response curve for mutations was essentially linear in the repair-deficient cells. In the normal cells, however, the curves for induced resistance to thioguanine and azaadenine were complex and were curvilinear with increasing slope at low doses. This behavior may be attributable to saturation of the excision repair system. No difference was seen in the efficiency of inducing ouabain-resistant mutations in the repair-deficient cells compared to the normal cells, indicating a qualitatively different behavior of this marker. These results are consistent with excision repair of 7-BrMeBA damage being error-free in Chinese hamster ovary cells. Sister chromatid exchange, another manifestation of DNA damage, also was induced with greater efficiency in the repair-deficient cells. (Author abstract) (34 Refs)

22. THE INDUCTION OF DNA DAMAGE AND ITS REPAIR IN YEAST AFTER EXPOSURE TO 4CMB, BC AND 4HMB.

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Dept. Genetics, Univ. College Swansea, Singleton Park, Swansea SA2 8PP, Wales
Mutat Res; 100(1-4):119-122 1982

DNA-damaging abilities of 4-chloromethylbiphenyl (4CMB), benzyl chloride (BC), a nd 4-hydroxymethylbiphenyl (4HMB) in yeast were examined using a modification of the alkaline sucrose sedimentation technic. 4HMB did not induce DNA damage. 4CMB and BC induced comparable DNA damage. The yeast DNA-repair mechanisms appeared able to repair a greater percentage of BC-induced lesions than 4CMB-induced lesions after a repair-incubation period. (2 Refs)

23. POST REPLICATION REPAIR IN AN EXCISION DEFECTIVE STRAIN OF ESCHERICHIA COLI FOLLOWING TREATMENT WITH CIS DICHLORODIAMMINEPLATINUM(II).

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Biochem Biophys Res Commun; 104(2):693-700 1981

Alkaline sucrose density gradient centrifugation was used to determine the size of the DNA synthesized after treatment of the excision-defective mutant AB 2500 uvr A6 of Escherichia coli with cis-dichlorodiammineplatinum(II) (cisPDD) at 5, 15, or 30 ug/ml for 1 hr. Exposure to UV light yielded DNA with a reduced mol wt of about 12 x 10(6) daltons, compared to 10(8) daltons for the control. A similar concentration-dependent effect on DNA size was evident after cis-PDD treatment, with DNAs of about 28 x 10(6) daltons being produced after 30 ug/ml cis-PDD treatment. During post-treatment incubation the size of DNA increased in cis-PDD-treated cultures as indicated by the sedimentation profile, suggesting that single strand gaps or breaks had been caused by treatment but were rejoined during post-treatment incubation. The reduction in DNA size caused by cis-PDD is probably due to a delayed replication caused by binding of the compound to DNA. The return toward control-sized DNA during post-treatment suggested that a process similar to post-replication repair was at work. (20 Refs)

24. REPAIR OF MITOCHONDRIAL DNA IN SACCHAROMYCES CEREVISIAE. INDUCTION OF CYTOPLASMIC PETITE MUTANTS IN A NUCLEAR MUTANT EXHIBITING THERMOSENSITIVE MITOCHONDRIAL DEOXYRIBONUCLEASE ACTIVITY.

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J Biol Chem; 257(2):781-787 1982

Four nuclear thermosensitive mutants have been obtained in which induction of up to 100% cytoplasmic petite mutants (rho-) is observed upon cell incubation at 36 C. For a given incubation time at 36 C, the percentage of rho- is increased by preliminary gamma-ray irradiation. Under these conditions, the induction of rho- is a linear function of the irradiation dose. The retention of genetic information by rho- and of mitochondrial DNA synthesis in vivo and in vitro exclude that the mutants are deficient in the replication of mitochondrial DNA. The degradation of mitochondrial DNA labeled with [3H]dTTP in isolated mitochondria, has been monitored at 26

C and at 36 C after addition of 0.5% Triton X-100 in the presence or in th e absence of ethidium bromide. In assays carried out at 26 C, the degradation of mitochondrial DNA is similar in the parental strain and in the mutant gamma s rho 2. However, at 36 C, the degradation of mitochondrial DNA is slower in the mutant. We have shown that a mitochondrial membrane deoxyribonuclease acting on double-stranded DNA at acid pH is thermosensitive in the mutant. Analysis of the meiotic segregants of a tetrad issued from the cross of the mutant with an isogenic parental strain shows co-segregation of rho- induction and of nuclease thermosensitivity in a 2:2 Mendelian pattern. These results suggest that a mitochondrial deoxyribonuclease is involved in the repair of damages caused to mitochondrial DNA by elevated temperature and by gamma-rays. (Author abstract) (29 Refs)

25. CHANGES IN THE SUPERCOILED STRUCTURE OF NUCLEAR DNA IN RAT AND HUMAN PERIPHERAL BLOOD LYMPHOCYTES AFTER GAMMA-IRRADIATION.

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Central Res. Inst. Rontgenology and Radiology, USSR Ministry Public Health, Leningrad 188646, USSR

Int J Radiat Biol; 41(2):221-225 1982

Damage and repair of DNA as indicated by changes in the supercoiled structure (SCS) of DNA were studied in vitro and in vivo. After exposure to gamma-irradiation DNA showed a relaxation of SCS. Both in vitro and in vivo DNA was repaired in a three-phase process reflecting repair of single-strand breaks, degradation of DNA, and further repair of single-strand breaks. The process was delayed in vivo, suggesting the presence of inhibiting factors. (21 Refs)

26. DNA REPAIR AFTER GAMMA RADIATION AND SUPEROXIDE DISMUTASE ACTIVITY IN LYMPHOCYTES FROM SUBJECTS OF FAR ADVANCED AGE.

Licastro F, Franceschi C, Chiricolo M, Battelli MG, Tabacchi P, Cenci M, Barboni F, Pallenzona D

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Carcinogenesis; 3(1):45-48 1982

DNA repair after gamma radiation was studied in purified T lymphocytes from young and aged subjects. Two different assays were employed. In the first, T lymphocytes were stimulated with phytohemagglutinin (PHA) for 72 hr and then treated with hydroxyurea, irradiated with 30 K rads and pulsed with [3H]thymidine (TdR) for 4 hr. In the second, T lymphocytes were first irradiated with graded doses of gamma rays (200-800 rads) and then stimulated with PHA, cultured for 72 hr and pulsed with 3H-TdR for the last 6 hr of culture. T lymphocytes from aged subjects showed a lack of DNA repair synthesis in the first assay whereas only minor differences were found in the second assay between the two groups, ie, a certain degree of radioresistance in aged lymphocytes. Lymphocyte superoxide dismutase activity showed great individual variations in both groups and a slight increase in old subjects. (Author abstract) (44 Refs)

27. THE SENSITIVITY OF ACTIVE AND INACTIVE CHROMATIN TO IONIZING RADIATION-INDUCED DNA STRAND BREAKAGE.

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Int J Radiat Biol; 41(1):71-77 1982

Sensitivity of Tetrahymena pyriformis strain GL-C DNA in actively transcribing and inactive states was compared with regard to gamma-radiation-induced single-strand break (SSB) induction. Following electrophoresis on agar gels and hybridization with poly(A+)RNA, nuclear DNA from irradiated cells was found in broader peaks and contained smaller fragments than did control DNA. In unirradiated cells, the profiles of transcribing and bulk DNA were similar, suggesting that transcribing genes are initially randomly distributed in all fragment sizes of nuclear DNA. Transcribing DNA from irradiated cells was found preferentially among the smaller fragments of total DNA. The apparent difference in the behavior of transcribing DNA from unirradiated and irradiated cells was not due to contamination of poly(A+)RNA with ribosomal RNA. The av mol wt of transcribing DNA was slightly but consistently greater than that of total nuclear DNA in unirradiated cells, while the reverse was true for irradiated DNA. When unirradiated nuclear DNA was mechanically sheared into fragment sizes comparable to those from irradiated cells, the similarity of the profiles of chromosomal and transcribing DNA was maintained after reduction of the fragment size of nuclear DNA. In vitro, no differences in the radiation sensitivities of transcribing and chromosomal DNA were observed. Kinetics of SSB rejoining are described. The results indicated that chromatin organization is important in the determination of the sensitivity of cellular DNA towards gamma-radiation. Not only yield but also the rate of repair of SSB is greater in actively transcribing genes than in the total nuclear DNA. (30 Refs)

28. RADIOIMMUNE ASSAY OF INDUCTION AND REMOVAL OF UV LESIONS IN TOTAL AND STAPHYLOCOCCAL NUCLEASE-RESISTANT DNA OF MAMMALIAN CHROMATIN.

McConlogue LC, Ward JF, Lewis HL, Norman A

Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143

Radiat Res; 89(2):381-395 1982

A direct lesion radioimmunoassay was used to measure induction and repair removal of UV lesions in total DNA (DNAt) and Staphylococcal nuclease-resistant DNA (DNAr). UV lesion induction in DNAr of HeLa and CHO cells was about 80% of that in DNAt. The two- to four-fold difference in repair synthesis observed in these DNAs cannot be accounted for by this result. The equal amounts of lesions per unit DNA in DNAr and DNAt of HeLa cells at 6 hr of repair induction can be explained by either the lower rate of lesion removal in staphylococcal nuclease-resistant DNA or complete nucleosomal rearrangement with respect to UV lesions during repair. (35 Refs)

29. DNA REPAIR AND MUTAGENESIS OF SINGLE-STRANDED BACTERIOPHAGES.

Doubleday OP, Brnadenburger A, Godson GN, Wagner R, Radman M
Rue des Chevaux 67, B-1640 Rhode-St-Genese, Belgium
Gann Monogr Cancer Res; 27:3-11 1982

DNA sequences of revertants of two M13 amber mutants, am6H2 and am7H3, isolated from populations of irradiated and unirradiated phages grown in irradiated or unirradiated host bacteria were analyzed. DNA sequences in the region of the amber mutation were determined. Forty-nine revertants of am6H1 and 125 of am7H3 were sequenced. All detectable single nucleotide changes were seen except the thymine-adenine-guanine (TAG) to GAG change, which was not found for either amber. No obvious specificity of mutagenesis by either UV- or gamma-irradiation of target DNA were seen. No specificity of mutagenesis by an error-prone repair activity (SOS repair system) acting upon either irradiated or unirradiated DNA was revealed, although induction of host SOS repair activity increased revertant yields. Both transitions and transversions, and changes involving each of the three bases of the amber codon were induced by all combinations of host cell and target irradiation. No pattern of revertant sequences was obtained to suggest that pyrimidine dimers are the primary sites of base changes. DNA sequences of revertants isolated from UV-irradiated phage populations demonstrated that pyrimidine dimers are not the main sites of mutations in UV-irradiated DNA. UV-induced mutagenesis may be untargeted or may occur at sites of lesions other than pyrimidine dimers. (23 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

30. IDENTIFICATION OF THYMIDINE-5'-ALDEHYDE AT DNA STRAND BREAKS INDUCED BY NEOCARZINOSTATIN CHROMOPHORE.

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Proc Natl Acad Sci USA; 79(3):744-748 1982

Snake venom phosphodiesterase or endonuclease S1 digestion of neocarzinostatin chromophore-treated DNA, labeled in its thymidine residues, liberates an unusual labeled nucleoside from the 5' end of a drug-induced break. This substance, isolated by reverse-phase HPLC, possesses carbons from both the thymine and the deoxyribose moieties of thymidine in the DNA but, unlike thymidine, is readily degraded at pH 12 to thymine and a sugar fragment. The altered nucleoside was shown to contain a carbonyl group by its reduction with NaBH4 to form a substance that has the chromatographic properties of thymidine and by its reaction with various hydrazines to form the respective hydrazone derivatives; the carbonyl exists as the 5' aldehyde as shown by its mild chemical oxidation to the carboxylic acid with simultaneous loss of the 5' 3H. Mass spectral analysis showed a fragmentation pattern compatible with the structure t hymidine-5'-aldehyde. These data indicate that the nonprotein chromophore of neocarzinostatin, in the presence of a reducing substance (2-mercaptoethanol) and molecular oxygen, selectively oxidizes the 5' carbon of nucleosides in DNA to the aldehyde, resulting in a strand break and a DNA fragment bearing nucleoside-5'-aldehyde at its 5' end. (Author abstract) (26 Refs)

31. COVALENT ADDUCTS OF DNA AND THE NONPROTEIN CHROMOPHORE OF NEOCARZINOSTATIN CONTAIN A MODIFIED DEOXYRIBOSE.

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Proc Natl Acad Sci USA; 79(2):369-373 1982

When the nonprotein chromophore of neocarzinostatin was allowed to react with either calf thymus DNA or poly(dA-dT)-poly(dA-dT) in the presence of 2-mercaptoethanol and the DNA was precipitated with ethanol, 5% of the fluorescence attributable to the naphthalene rings of the chromophore coprecipitated with the DNA. Most of this fluorescence remained attached to DNA through successive reprecipitations, suggesting formation of covalent adducts between chromophore and DNA. Enzymatically digested poly(dA-dT)-poly(dA-dT)-chromophore adduct contained, in addition to deoxyadenosine and thymidine, several highly fluorescent hydrophobic products, separable by reverse-phase chromatography, all of which contained both adenine and thymine radiolabel, as well as chromophore radiolabel. One such product consistently had twice as much thymine as adenine, suggesting a structure chromophore-d(TpApT), in which the attached chromophore rendered both phosphodiester bonds refractory to endonuclease S1. This adduct fragment was completely hydrolyzed at pH 12, releasing adenine, 3'-dTMP, and 5'-dTMP. At pH 7, the adduct fragment slowly released chromophore and 3'-dTMP with parallel kinetics, leaving a modified d(ApT), which was cleaved by snake venom phosphodiesterase to yield 5'-dTMP and a modified deoxyadenosine. These hydrolysis patterns are unlike those of any previously characterized base or phosphotriester DNA adduct but rather indicate an altered deoxyadenosine sugar. The formation of adducts containing a modified deoxyribose suggests that deoxyribose may be the site of covalent chromophore attachment. Alteration of this same site, possibly the 5'-carbon of the sugar moiety, may account for the extreme lability of the phosphodiester bond. (Author abstract) (25 Refs)

32. DEGRADATION OF HELA CELL CHROMATIN BY NEOCARZINOSTATIN AND ITS CHROMOPHORE.

McHugh MM, Woynarowski J, Beerman T
(c/o Beerman) Dept. Experimental Therapeutics, Roswell Park Memorial Inst., 666 Elm St., Buffalo, NY, 14263
Biochim Biophys Acta; 696(1):7-14 1982

Chromatin is the in vivo target site for neocarzinostatin, a DNA strand scission antitumor drug. The effect of neocarzinostatin and its active chromophore component on HeLa cell chromatin is described here. Chromatin consisting of a mixture of mono-, di-, tri- and larger nucleosome fragments is prepared by micrococcal nuclease digestion of HeLa cell nuclei. Drug-induced conversion of chromatin to smaller sized fragments is measured by electrophoresis of the DNA on non-denaturing 4% polyacrylamide gels. Chromatin breakdown measured under these conditions is double-stranded in nature. In the presence of 2 mM dithiothreitol, neocarzinostatin causes degradation of large chromatin fragments and a loss of distinct nucleosome peaks. Detection of chromatin breakdown by neocarzinostatin is dependent upon the concentration of chromatin in the assay. When chromatin is increased from 14 to 70 ug/ml, changes in the larger fragments caused by 100 ug/ml neocarzinostatin become less obvious and are almost undetectable at 140 ug/ml chromatin. No change is observed when chromatin is treated with either neocarzinostatin or its chromophore in the absence of dithiothreitol. For detectable levels of chroma-

tin degradation, 10 ug/ml neocarzinostatin is required compared to only 2.5 ug/ml chromophore (expressed in ug equivalent neocarzinostatin). Such degradation also occurs more rapidly with chromophore than with neocarzinostatin. Digestion of chromatin with neocarzinostatin continues for at least 30 min at 37 C, while similar degradation caused by chromophore is complete in 1 min. Neocarzinostatin levels which actively degrade isolated chromatin can also effect release of soluble chromatin from intact nuclei. The released chromatin can serve as a substrate for micrococcal nuclease digestion. Such chromatin studies should prove useful in characterizing the mechanism of action of DNA reactive drugs such as neocarzinostatin. (Author abstract) (29 Refs)

33. CHARACTERISTICS OF ENZYMATIC DNA METHYLATION IN CULTURED CELLS OF HUMAN AND HAMSTER ORIGIN, AND THE EFFECT OF DNA REPLICATION INHIBITION.

Woodcock DM, Adams JK, Cooper IA
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Biochim Biophys Acta; 696(1):15-22 1982

In mammalian cells, inhibitors of DNA replication have been shown to induce chromosomal aberrations, cell death and changes in gene control. Inhibition of DNA synthesis has been reported to induce hypermethylation of mammalian DNA (enzymatic postsynthetic formation of 5-methylcytosine). These 5-methylcytosines in mammalian DNA have variously been suggested to be important in gene control, DNA repair, and control of DNA replication. In establishing the normal characteristics of enzymatic DNA methylation, we have demonstrated that, in asynchronously growing cells of both human and hamster origin, some cytosine methylation is delayed for several hr after strand synthesis and that this delayed methylation is completed before the DNA strand acts as a template for DNA replication in the next S-phase. Further, in testing whether the deleterious effects on mammalian cells of DNA synthesis inhibitors might be mediated via changes in enzymatic DNA methylation, we have found, contrary to some previous findings, no evidence for any change in the level of DNA methylation in DNA strands synthesized during 6 hr of treatment of cells of human origin with high concentrations of four different inhibitors of DNA replication or during the 4 hr following the 6 hr treatment. Almost totally blocking DNA replication had no effect on the small amount of delayed methylation of DNA strands not involved in semi-conservative replication during the time of the experiment. This lack of effect on DNA methylation was obtained when the labeling medium contained normal, undialyzed serum. In contrast, if dialyzed serum was used in the labeling medium in order to maximize L-[Me-3H]methionine utilization, highly variable, totally irreproducible patterns of apparent DNA hypermethylation were obtained. (Author abstract) (27 Refs)

34. IN VIVO DAMAGE TO DNA: SEDIMENTATION STUDIES OF GIANT DNA MOLECULES FROM SOMATIC TISSUES.

Appleby DW, Appleby NH
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Ecotoxicol Environ Saf; 5(4):443-451 1981

A method for detecting giant DNA molecules based on centrifugation at low speed for various periods of time to define the distribution of sedimentation coefficients in a cellular lysate is described. The lysate is constructed so as to preserve in vivo mol wt of DNA molecules. DNA molecules are separated in a centrifuge tube run in the preparative ultracentrifuge. The tube is then fractionated to determine the positions occupied by the DNA. Data collected on Escherichia coli and Drosophila cells are reviewed. The major problem with application of the method to tissue biopsies is that DNA cannot be labeled in vivo to a sufficiently high specific activity. However, DNA can be detected after running the gradients by passing the individual fractions through nitrocellulose filters after denaturing the DNA. DNA bound to nitrocellulose and fixed can then be challenged with a short DNA sequence labeled to high specific activity in vitro. Short DNA sequences can then be identified. Use of this method with bacteriophage lambda DNA and the mouse satellite sequence is described. Application of the technique as a specific probe for the centromeres of chromosomes and for the whole chromosome may be possible. (11 Refs)

35. REARRANGEMENTS OF DNA-PROTEIN INTERACTIONS IN ANIMAL CELLS COUPLED WITH CELLULAR GROWTH-QUIESCENCE TRANSITIONS.

Lichtenstein AV, Sjakste N, Zaboykin MM, Shapot VS
All-Union Oncological Scientific Center, Moscow 115478, USSR
Nucleic Acids Res; 10(3):1127-1145 1982

Overall DNA-protein interactions in animal cells undergo drastic changes coupled with cellular transitions from quiescence to growth and reversely as revealed by nucleoprotein-Celite chromatography. DNA of chromatin was found to exist in one of the two sharply distinct alternative forms, namely, either tightly or weakly bound to protein moiety. These forms are specific for cycling and quiescent cells, respectively The tight DNA-protein interactions characterize all cycling cells independent of the cell cycle phase. Transition of DNA of cycling cells from one form to another was observed as a result of treatment of isolated nuclei with DNase I. (Author abstract) (18 Refs)

36. ISOLATION AND CHARACTERIZATION OF CHICKEN DNA HOMOLOGOUS TO THE TWO PUTATIVE ONCOGENES OF AVIAN ERYTHROBLASTOSIS VIRUS.

Vennstrom B, Bishop JM
Dept. Microbiology, Univ. Uppsala, Biomedical Center, Uppsala, Sweden
Cell; 28(1):135-143 1982

The genome of avian erythroblastosis virus contains two independently expressed genetic loci (v-erbA and v-erbB) whose activities are probably responsible for oncogenesis by the virus. Both loci are closely related to nucleotide sequences found in the DNA and RNA of chickens and other vertebrates. We have isolated and characterized chicken DNA homologous to v-erbA and v-erbB. The two viral genes are represented by separate domains within chicken DNA (c-erbA and c-erbB), which are separated by a minimum of 12 kilobases (kb) of DNA and may not be linked at all. The nucleotide sequences shared by the viral and cellular erb loci are colinear, but the cellular loci are interrupted by multiple intervening sequences of various lengths. Polyribosomes prepared from normal chicken embryos contain two polyadenylated RNAs transcribed from c-erbA and two transcribed from c-erbB. The evident coding regions of these RNAs represent an unusually small fraction of the lengths of the RNAs, as if the 3' untranslated domains of the RNAs might be exceptionally large (3-11 kb). These findings indicate that the c-erb loci are normal vertebrate genes rather than genes of cryptic endogenous retrovir-

uses, and that they may have a role in the metabolism of normal cells. It appears that the viral erb genes, like most other retrovirus oncogenes, have been copied from cellular genes. In the viral genome, the two genes are devoid of introns, but they remain independently expressed loci, and they remain colinear with the coding domains of their cellular progenitors. (Author abstract) (30 Refs)

37. STRUCTURAL RELATIONSHIP BETWEEN A NORMAL CHICKEN DNA LOCUS AND THE TRANSFORMING GENE OF THE AVIAN ACUTE LEUKEMIA VIRUS MC29.

Robins T, Bister K, Garon C, Papas T, Duesberg P
Dept. Molecular Biology, Univ. California, Berkeley, CA, 94720
J Virol; 41(2):635-642 1982

We screened a recombinant chicken DNA/lambda phage library for sequences homologous to the transformation-specific sequences of the avian acute leukemia virus MC29 by hybr idization with molecularly cloned MC29 proviral DNA. Three cellular DNA clones were found and compared with each other and with the viral genome by physical mapping with restriction endonucleases and by heteroduplex analysis. These experiments indicated that the three cellular clones overlap and represent a single cellular locus. The RNA genome of MC29 and normal cell DNA share a homologous region of 1.6 kilobases which is interrupted in the cellular DNA by 1.0 kilobase of sequences not present in the viral genome. Hybridization of the cloned cellular DNA to viral RNA and analysis of the protected viral RNA by fingerprinting techniques indicated that there is extensive sequence homology between the helper virus-unrelated mcv sequences of the viral RNA and the cellular DNA, with only minor base differences. The cellular mcv locus, however, lacks all helper virus-related sequences of MC29, including those of the partial viral gag gene which, together with mcv, encodes the probable transforming protein of MC29. We conclude that although the mcv locus of the normal cell does not represent a complete structural homolog to the onc gene of MC29, it is probably the precursor to the onc-specific sequence in the virus. (Author abstract) (27 Refs)

38. ON IMMUNOGLOBULIN HEAVY CHAIN GENE SWITCHING TWO 5G 12B GENES ARE REARRANGED VIA SWITCH SEQUENCES IN MPC-11 CELLS BUT ONLY ONE IS EXPRESSED.

Lang RB, Stanton LW, Marcu KB
Biochemistry Dept., State Univ. New York at Stony Brook, Stony Brook, NY, 11794
Nucleic Acids Res; 10(2):611-630 1982

During B lymphocyte differentiation, switches in the expression of heavy chain immunoglobulin constant region (CH) genes occur by a novel DNA recombination mechanism. We have investigated the requirements of the CH gene switch by characterizing two rearranged gamma2b genes from a gamma2b producing mouse myeloma (MPC-11). One of the two gamma2b genes is present in two-three copies per cell (gamma2b strong hybridizer) while the other is present in approx one copy per cell (gamma2b weak hybridizer). Genomic clones of the gamma2b strongly hybridizing gene indicate that this is an abortive switch event between the Sgamma3 and Sgamma2b regions. However, clones of the gamma2b weakly hybridizing gene suggest a functional rearrangement due to the presence of VH, JH and Smu sequences. The switch-recombination sites of these rearranged gamma2b genes and those of other CH genes show a high degree of preference for the sequence AGGTTG 5' of either the Smu donor site or the appropriate CH S acceptor site. AGGTTG and its analogs are rare in the Smu region, are somewhat prevalent in Salpha and in the case of Smu are found 5' of a tandemly repeated DNA sequence (GAGCT, GGGGT) comprising most of Smu. (Author abstract) (53 Refs)

39. REGULATION OF CASEIN GENE EXPRESSION IN HORMONE-DEPENDENT MAMMARY CANCER.

Rosen JM, Supowit SC, Gupta P, Yu LY, Hobbs AA, Dao TL, Topp, Hilf
Dept. Cell Biology, Baylor Coll. Medicine, Houston, TX, 77030
Banbury Rep; 8:397-424 1981

Structure, organization, and expression of the casein gene (CG) family and the alpha-lactalbumin gene in the normal mammary gland, and altered regulation of CG expression and gene expression in general in hormone-dependent mammary cancer, were studied. Milk protein structural genes, hormonal regulation of milk protein messenger RNA accumulation in rat mammary gland organ culture, milk gene organization and chromosomal localization, CG expression in hormone-dependent mammary cancer, methylation patterns of the CG, DNA rearrangement in mammary tumors (MT), and expression of unique single-copy DNA sequences in 7,12-dimethylbenz(a)anthracene-induced MT are reviewed. New approaches may help explain the regulation of differentiated function in the normal mammary gland and deviation of the mechanisms in hormone-dependent breast cancer. (38 Refs)

40. ISOLATION OF TRANSFORMING DNA BY COSMID RESCUE.

Lund T, Grosveld FG, Flavell RA
Lab. Gene Structure and Expression, Natl. Inst. Medical Res., The Ridgeway, Mill Hill, London NW7 1AA, England
Proc Natl Acad Sci USA; 79(2):520-524 1982

A procedure has been developed to allow the recovery of an integrated plasmid genome from a transformed cell, together with large areas of the flanking DNA sequences. DNA from Saccharomyces cerevisiae BAS2, in which the pBR322-ura 3 plasmid (Ylp5) is integrated at the yeast histone H2A and H2B locus, was used to generate a cosmid library, using a new cosmid vector (pTL5) that is ampicillin sensitive and tetracycline resistant. Colonies were selected for ampicillin resistance, which was conferred by the incorporation of the integrated pBR322 beta-lactamase gene into the recombinant cosmid. Restriction enzyme and blot hybridization analyses show that the rescued clones contain the yeast histone genes in addition to the Ylp5 sequences; a total of approx 50 kilobase pairs of DNA sequences flanking the plasmid was recovered as a series of overlapping cosmids. This approach should allow the recovery of most genes that can be linked to a marker pBR322 sequence and for which a specific phenotype can be selected in a recipient eukaryotic cell. (Author abstract) (15 Refs)

41. CDNA CLONES CODING FOR THE HEAVY CHAIN OF HUMAN HLA-DR ANTIGEN.

Lee JS, Trowsdale J, Bodmer WF
Imperial Cancer Res. Fund, Lincoln's Inn Fields, London WC2A 3PX, England
Proc Natl Acad Sci USA; 79(2):545-549 1982

Two cDNA clones, pDRH1 and pDRH2, containing sequences specific for human HLA-DR antigens were isolated from a bank of cDNA clones made from partially purified HLA-DR mRNA from the human lymphoblastoid cell line

Maja. The clones were specific for the Mr 34,000 HLA-DR antigen glycoprotein chain. Th e identity of these clones was established by (i) their ability to hybridize specifically to HLA-DR mRNA in a positive selection assay; (ii) mRNA species hybridizing to the cDNA clones were expressed in B-cell but not in T-cell or fibroblast cell cultures; and (iii) a nucleotide sequence in the longer clone, pDRH2, could be translated into an amino acid sequence that is identical to the limited NH2-terminal amino acid sequence available for the purified HLA-DR antigen Mr 34,000 chain. Analysis of dNA from human, mouse, and human-mouse somatic cell hybrid lines by Southern transfer of restriction endonuclease digests indicated that the HLA-DR heavy chain is encoded in chromosome 6. This finding is compatible with the location of at least one of the HLA-D/DR heavy chain genes within the HLA region. In addition, the sequences coding for HLA-DR heavy chain appear to be present in only one or a few copies in the genome and to be relatively simple in structure. (Author abstract) (43 Refs)

42. DNA SEQUENCE OF A GENE ENCODING A BALB/C MOUSE LD TRANSPLANTATION ANTIGEN.

Moore KW, Sher BT, Sun YH, Eakle KA, Hood L
(c/o Hood) Div. Biology, California Inst. Technology, Pasedena, CA, 91125
Science; 215(4533):679-682 1982

The sequence of a gene, denoted 27.5 encoding a transplantation antigen for the BALB/c mouse has been determined. Gene transfer studies and comparison of the translated sequence with the partial amino acid sequence of the Ld transplantation antigen establish that gene 27.5 encodes an Ld polypeptide. A comparison of the gene 27.5 sequence with several complementary DNA sequences suggests that the BALB/c mouse may contain a number of closely related L-like genes. Gene 27.5 has eight exons that correlate with the structural domains of the transplantation antigen. (Author abstract) (22 Refs)

43. INDUCTION OF PETITE FORMATION IN SACCHAROMYCES CEREVISIAE BY EXPERIMENTAL ANTITUMOUR AGENTS. STRUCTURE-ACTIVITY RELATIONSHIPS FOR 9-ANILINOACRIDINES.

Ferguson LR, Baguley BC
Cancer Res. Lab., Univ. Auckland Medical Sch., Private Bag, Auckland, New Zealand
Mutat Res; 90(4):411-423 1981

Derivatives of 9-anilinoacridine (9AA) with unsubstituted or substituted acridine rings at the 3 and/or 6 position were tested for inhibition of growth of L1210 murine leukemia cells in vitro and induction of respiration-deficient (petite) mutations in D5 diploid Saccharomyces cerevisiae. D5 cells were exposed to test compounds in growth and nongrowth conditions. Comparison of the effects of the petite-inducers ethidium (ETH) and proflavine (PRO) showed that ETH converted at least 99% of D5 cultures to petite over a wide range of doses; PRO did not exceed 90% at any dose level. PRO produced petites at doses 120x those of ETH and only at cytotoxic doses. Tests of petite induction (PI) by various 9AA showed three c lasses of compounds: inactive, ETH-like, and PRO-like. The existence of two active classes suggests that two different targets involving DNA mediate the effects of 9AA. At least one acridinyl amino group was required for induction of petites. Amino groups increased DNA binding. Small anilino substituents on 3-amino-9AA produced PI similar to that of PRO; larger substituents abolished all PI. 3,6-Diamino 9AA had PI similar to that of ETH. Max PI did not correspond to max inhibition of L1210 cells. Results indicate that DNA binding and biological activity in both D5 and L1020 systems are related to the size of anilino substituents. (18 Refs)

OTHER RELATED STUDIES

44. THE NATURE OF CHROMOSOME BANDS AND THEIR SIGNIFICANCE FOR CANCER RESEARCH.

Sumner AT
Clinical and Population Cytogenetics Unit, Western General Hosp., Edinburgh EH4 2XU, Scotland
Anticancer Res; 1(4):205-215 1981

Chromosome bands are discussed with respect to classification, properties, banding techniques, and their significance to cancer research. The two main classes of chromosome bands are constant, heterochromatic bands (CHB) and fluctuant bands (FB), with kinetochores and nucleolar organizers recognized as separate classes. CHB have been associated with regions containing satellite DNA in many species. FB correspond to pachytene chromomeres, and may represent the condensation process occurring in mitotic chromosomes. Banding patterns are used to identify whole or parts of chromosomes, gene mapping, and to study the clinical significance of polymorphisms of CHB. Banding techniques revealed the presence of the Philadelphia chromosome in most cases of chronic myeloid leukemia; few, if any, other cancers show such a clear correlation with a specific chromosomal abnormality. Banding techniques have revealed that cancers tend to be associated with particular chromosome abnormalities and that certain chromosomes tend to be altered in cancerous cells. Burkitt's lymphoma retinoblastoma, and Wilm's tumor are each associated with specific chromosome abnormalities. The presence of 'homogenously staining regions' and double minute chromosomes in some tumor cells is discussed. While chromosome changes in cancer cells can be identified by banding techniques, the significance of such changes is unclear. (131 Refs)

45. THE RAT XC SARCOMA CELL LINE: RIBOSOMAL RNA GENE AMPLIFICATION AND BANDED KARYOTYPE.

Tantravahi U, Erlanger BF, Miller OJ
(c/o Miller) Dept. Human Genetics and Development, Coll. Physicians and Surgeons, Columbia Univ., 701 West 168th St., New York, NY, 10032
Cancer Genet Cytogenet; 5(1):63-73 1982

Ribosomal RNA gene amplification has been demonstrated in the rat XC sarcoma cell line. Cells of this rat line have a fairly stable karyotype with several unusual features, which have be en clarified by in situ hybridization, silver staining, and binding of antibodies to 5-methylcytosine. There are one or two tiny acrocentric chromosomes containing transcriptionally active 18S and 28S ribosomal RNA (rRNA) genes. The short arm of one chromosome No 12 has been replaced by a GC-rich, C-band negative, differentially staining region (DSR) containing an increased number of rRNA genes; most of these are transcriptionally inactive and located in regions containing highly methylated DNA. The short arm of a small chromosome, probably a No 20, has been replaced by a GC-rich, C-band negative, homogenously staining region (HSR) that presumably represents amplification of a DNA sequence other than the 18S and 28S rRNA coding sequences. The DNA in this HSR is not enriched in 5-methylcytosine and neither is

that in the HSRs of methotrexate-resistant Syrian hamster cells. (Author abstract) (40 Refs)

46. THE DISTRIBUTION OF INDUCED SISTER-CHROMATID EXCHANGES: A TOOL FOR IDENTIFYING AGENTS DIRECTLY INTERACTING WITH DNA.

Rainaldi R, Mariani T
Istituto di Mutagenesis DIfferenziamento CNR, Via Svegia 10, 56100-Pisa, Italy
Mutat Res; 103(3-6):333-337 1982

Exponentially-growing V79-AP4 Chinese hamster cells were treated for 1 hr with the direct alkylating agent ethyl methanesulfonate (EMS: 1) and 10 milliM and the DNA-synthesis inhibitors 5-bromodeoxyuridine (BUdR: 0.4, 0.8, 1.6 milliM) and aphidicholine (APC: 5, 10, 20 ug/ml). THe frequency distributions of sister-chromatid exchanges (SCEs) were not changed after EMS, although the mean number of SCEs increased with concentration. With BUdR and APC the median SCE/cell was about the same for all concentrations, but the spread of the frequency distribution was markedly increased as concentrations of both agents increased. Comparing SCE distribution patterns in this manner makes it possible to determine whether SCEs were induced by factors inside DNA (damaged template, EMS) or outside DNA (damaged replication machinery, BUdR, APC). (11 Refs)

47. OXYGEN DEPENDENCE OF SISTER-CHROMATID EXCHANGES.

Gutierrez C, Lopez-Saez JF
Instituto de Biologia Celular, Velazquez 144, Madrid-6, Spain
Mutat Res; 103(3-6):295-302 1982

Root meristems of Allium cepa L were subjected to gas mixtures of 5, 10, 20, and 100% oxygen pressure to observe the effect of oxygen on subsequent 5-bromodeoxyuridine (BrdUrd)-induced sister-chromatid exchanges. Onion roots previously grown at a fixed oxygen pressure were allosed to replicate in the presence of BrdUrd for either one or two cycles. SCE frequencies were unchanged at oxygen pressure of 20% (air) or below, but increased at higher levels. The mechanism responsible for BrdUrd-induced SCE formation appears to operate by exchanging postreplicative DNA portions, with activation of newly incorporated B rdUrd in the formation of SCEs. (20 Refs)

48. DNA PATTERN AND CYTOLOGICAL FINDINGS IN FINE-NEEDLE ASPIRATES OF UNTREATED PROSTATIC TUMORS. A FLOW-CYTOFLUOROMETRIC STUDY.

Ronstrom L, Tribukait B, Esposti PL
(c/o Tribukait) Dept. Radiobiology Medical, Karolinska Institutet, S-104 01 Stockholm, Sweden
Prostate; 2(1):79-88 1981

Flow-cytofluorometric analysis was used to determine DNA distribution patterns in 500 patients (50-92 yr old; mean age 71 yr) with clinical suspicion of carcinoma of the prostate. Fluorescence of each cell, proportional to nuclear DNA content, was measured at a rate of up to 1,000 cells/sec. Diploid or nondiploid (tetraploid or non-tetraploid) cell populations were identified. Of 301 benign lesions, 278 were diploid, 22 tetraploid, and 1 non-tetraploid. Of 33 suspected carcinomas, 24 were diploid, 6 tetraploid, and 3 non-tetraploid. Of 166 carcinomas, 45 were diploid, 75 tetraploid, and 46 non-tetraploid. Of the 41 well-differentiated carcinomas, 23 were diploid, 17 tetraploid, and 1 non-tetraploid; these values were 21, 52, and 22 for 95 moderateley differentiated carcinomas, and 1, 6, and 23 for 30 poorly differentiated carcinomas, respectively. Thus, differences in nuclear DNA content were observed in morphologically indistinguishable tumors allowing a further subdivision and characterization. Clinical follow-up of patients is necessary to determine the biological significance of these findings. (28 Refs)

49. COUPLING OF CHROMATIN STRUCTURE TO CELL GEOMETRY DURING THE CELL CYCLE: TRANSFORMED VERSUS REVERSE-TRANSFORMED CHO.

Nicolini C, Beltrame F
Dept. Physiology/Biophysics, Temple Univ. Sch. Medicine, 3223 North Broad, Philadelphia, PA, 19140
Cell Biol Int Rep; 6(1):63-71 1982

The effect of nuclear and cellular geometry on proliferation and DNA synthesis was investigated in transformed and fibroblast-like Chinese hamster ovary (CHO) cells. It was found that while nuclear morphometry and cell morphometry in homogeneous phases of cell growth were coupled for normal-like (cAMP reverse-transformed) cells, they were uncoupled for transformed CHO cells. (12 Refs)

50. ANTIBODIES SPECIFIC FOR DNA COMPONENTS STRUCTURALLY MODIFIED BY CHEMICAL CARCINOGENS.

Muller R, Rajewsky MF
(c/o Rajewsky) Institut fur Zellbiologie (Tumorforschung), Universitat Essen (GH), Hufelandstrasse 55, Essen 1, W. Germany
J Cancer Res Clin Oncol; 102(2):99-113 1981

Preparation, properties, and applications of antibodies against carcinogen-DNA adducts are reviewed. Specific topics discussed are procedures for the coupling of carcinogen-modified nucleosides (haptens) to carrier proteins, immunization procedures and carrier proteins used for antibody production, assays of antibody specificity and affinity, quantitative immunologic assays of carcinogen-modified DNA components, and use of the antibodies in in vivo and cell culture experiments. (69 Refs)

51. CYTOTOXICITY OF L-CANAVANINE IN VITRO.

Naha PM
Dept. Cell Biology, Christie Hosp. and Holt Radium Inst., Paterson Labs., Manchester M20 9BX, England
Anticancer Res; 1(5):275-277 1981

Cytostatic cellular reactions caused by L-canavanine (CAN) were studied in BALB/c-3T3 cells and phytohemagglutinin-stimulated human lymphocytes. BALB/c-3T3 cells were exposed to L-arginine (ARG) or CAN at concentrations of 1 x 10(-4) M, 1 x 10(-5) M, or 5 x 10(-6) M for 48 hr. Human lymphocytes were exposed to CAN at 1 x 10(-4) M to 1 x 10(-6) M for 50 hr at 37 C, then mitotic indices, karyotypes, and sister chromatid exchanges were studied. Of BALB/c-3T3 cells exposed to CAN for 48 hr then placed in growth medium without CAN, 90% treated with up to 1 x 10(-4) M recovered to continue DNA synthesis within 24 hr. Compared with ARG-treated cells, high labeling indices were observed after withdrawal of CAN, probably due to partial synchrony caused by CAN-induced inhibition of DNA synthesis. At lower concentrations (1 x 10(-6) M), the lower labeling index suggested partial inhibition and poorer synchrony. Cells exposed to 1 x 10(-4) M CAN for 48 hr recovered to form colonies as efficiently as untreated controls. In the presence of CAN, however, there was total inhibition of colony-forming ability. In the

presence of CAN, few phytohemagglutinin-stimulated lymphocytes proceeded with mitosis, while mitosis continued when CAN (1 x 10(-4) M) was withdrawn. No difference in chromosomal aberrations or sister chromatid exchanges were noted between CAN treated and control cultures. (17 Refs)

52. TRNA PRECURSOR TRANSCRIBED FROM A MUTANT HUMAN GENE INSERTED INTO A SV40 VECTOR IS PROCESSED INCORRECTLY.

Zasloff M, Santos T, Hamer DH
Genetics and Biochemistry Branch, Natl. Inst. Arthritis, Diabetes, Digestive and Kidney Diseases, NIH, Bethesda, MD, 20205
Nature; 295(5849):533-535 1982

Functional properties of the human variant initiator transfer (t)RNA gene within an intact cell were assayed by studying its transcription from a simian virus 40 (SV40) recombinant during lytic infection of African green monkey kidney cells. Structure of the SV40-tRNAiMet recombinant (SVtm) used contained a 1.15-kilobase fragment of human genomic DNA harboring a single tRNAiMet gene. Resulting viral stocks were used in the study. Total cytoplasmic and nuclear RNA were recovered from the infected cells and analyzed. Cytoplasmic and nuclear fractions from SVtm-infected cells contained a single low-mol wt species approx 89 nucleotides long. The RNA species accumulating in the SVtm infection was analyzed by classical fingerprinting techniques. The transcript was a precursor of the variant tRNAiMet. Only a preliminary analysis of base modification within the precursor was performed. Hybridization of equal amounts of gel-purified tRNA from SVtm and control infections to filter bound tRNAiMet DNA occurred at approx equal rates and reached max level of hybridization of approx 1% of the input radioactivity. Results indicate that conversion of the mutant precursor to mature tRNA does not occur with any significant efficiency, suggesting that mutations within eukaryotic tRNA genes may be more prevalent than assumed from the limited sequence variation observed in mature tRNA species. (22 Refs)

53. ISOLATION OF MUTANT PROMOTERS IN THE ESCHERICHIA COLI GALACTOSE OPERON USING LOCAL MUTAGENESIS ON CLONED DNA FRAGMENTS.

Busby S, Irani M, De Crombrugghe B
Departement de Biologie Moleculaire, Institut Pasteur, 25 Rue du Docteur Roux, 75724 Paris Cedex 15, France
J Mol Biol; 154(2):197-209 1982

We have worked out a system to obtain mutations that map in the promoter region of the Escherichia coli galactose operon. In order to easily detect small changes in gal promoter activity, we constructed a plasmid containing an operon fusion in which the lactose operon structural genes were controlled by the galactose operon promoter region. In cells harboring this plasmid, even modest variations in the expression of the lac genes could be detected on MacConkey lactose indicator plates. Enrichment for mutations that map in the promoter segment of the galactose operon was achieved by mutagenesis in vitro of a small fragment of DNA covering the promoter region. After insertion of the mutagenized gal promoter fragment into the gal-lac fusion plasmid, lac- cells were transformed and screened for an altered Lac+ phenotype on indicator plates. Several mutants were isolated due to lesions mapping in the small fragment covering the galactose promoter. In these mutants, the level of beta-galactosidase was between 15 and 50% of the wild-type level. The mutant promoters were subsequently reinserted into a plasmid containing the intact galactose operon. Cells harboring such plasmids, reconstituted with mutant galactose promoters, contained decreased levels of galactokinase that paralleled the decreases in beta-galactosidase. The biochemical properties of these mutants are reported in the accompanying paper. (Author abstract) (23 Refs)

54. SEPARATION OF MAJOR AND MINOR DEOXYRIBONUCLEOSIDE MONOPHOSPHATES BY REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: A SIMPLE METHOD APPLICABLE TO QUANTITATION OF METHYLATED NUCLEOTIDES IN DNA.

Christman JK
Dept. Biochemistry and Pediatrics, Mount Sinai Medical Center, 1 East 100th St., New York, NY, 10029
Anal Biochem; 119(1):38-48 1982

A simple reverse-phase chromatographic system for separating deoxyribonucleoside monophosphates is described. Using isocratic elution at room temperature, clear separation of seven of the de oxyribonucleoside monophosphates that occur in either prokaryotic or eukaryotic DNAs can be achieved in less than 2 hr. Thus, this method allows a sensitive and rapid analysis of submicrogram quantities of 32P-labeled deoxyribonucleoside monophosphates derived from DNA labeled in vivo with 32Pi or from DNA labeled enzymatically in vitro at the 5' or 3' ends. The suitability of the method for studying methylation of mammalian DNAs is illustrated by presenting examples of its application to (a) quantitation of major and minor nucleotides in newly synthesized DNA, (b) determination of the specificity of in vitro methylation of DNA, and (c) quantitation of the extent to which specific restriction endonuclease sites are methylated in vivo. (Author abstract) (28 Refs)

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MECHANISMS OF DNA REPLICATION

1. SITE-SPECIFIC MUTAGENESIS BY ERROR-DIRECTED DNA SYNTHESIS.

Zakour RA, Loeb LA
(c/o Loeb) Joseph Gottstein Memorial Cancer Res. Lab., Dept. Pathology,, SM-30 Univ. Washington, Seattle, WA, 98195
Nature; 295(5851):708-710 1982

The use of misincorporation during DNA synthesis to create site-specific mutagenesis was attempted using single-stranded bacteriophage phiX174 circular DNA containing an amber mutation. The fragment HpaI2 (phiX174 positions 31-1,294) was obtained from phiX174 RF DNA by endonuclease cleavage. The 3'OH terminus of the fragment was elongated using DNA polymerase from Escherichia coli with deoxyATP (dATP) and

HE 20.3172/2:

deoxycytosine triphosphate (dCTP), which were complementary to the first three template positions. A second synthesis phase utilized the error-prone DNA polymerase from avian myeloblastosis virus (AMV). Deoxynucleosides were used in three different combinations. After allowing for misincorporation, the subsequent correct nucleotide was inserted. The newly copied DNA was transfected into E coli, and the frequency of am18 mutations was determined. When dATP, which was complimentary to position 23 in the am18 region, was present during replication with AMV DNA polymerase, the reversion frequency was 4x that of uncopied template DNA. When dATP was not present, the reversion frequency was 100x times that of uncopied template DNA. Further experiments confirmed that reversions were caused by single-base substitutions. Thymine-guanine mispairing was more likely than thymine-thymine or thymine-cytosine mispairing. Results indicate that misincorporation can be used to create site-specific mutagenesis. (23 Refs)

EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

2. HORMONAL REGULATION OF NET DNA SYNTHESIS IN MCF-7 HUMAN BREAST CANCER CELLS IN TISSUE CULTURE.

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Cancer Res; 42(5):1727-1735 1982

Estrogens and anti-estrogens have striking effects on growth of target tissues in vivo. In order to examine these effects in vitro, experimental methods for the rigorous assessment of rates of net DNA synthesis in tissue culture systems are required. The quantitation of DNA synthesis with inorganic [32P]orthophosphate is described. The limitations of more conventional [3H]thymidine labeling are illustrated in this hormonally responsive system. Estrogen administration increases the specific activity of acid-soluble phosphate and increases net DNA synthesis relative to controls in MCF-7 human breast cancer cells. Stimulation is most evident after 36 hr of hormone treatment. Conversely, tamoxifen produces substantial inhibition of net DNA synthesis after 36 hr of hormone treatment. Thymidine availability modulates the effect of estrogens and anti-estrogens on DNA synthesis and constitutes an independent experimental variable. (Author abstract) (55 Refs)

3. INFLUENCE OF ESTRADIOL ON THE TOTAL UPTAKE AND INCORPORATION OF THYMIDINE IN HUMAN BREAST CANCER (MCF-7) IN LONG-TERM CELL CULTURE: COMPARISON OF AUTORADIOGRAPHIC AND BIOCHEMICAL METHODS.

Coosen R, De Jong PW, Schwarz F
Dept. Endocrinology, Univ. Hosp., 3511 GV Utrecht, The Netherlands
Mol Cell Biochem; 42(3):155-160 1982

The total uptake and incorporation into DNA of 3H-thymidine in MCF-7 cells is stimulated by estradiol after an incubation period of 24 hr. Prolonged incubations show a decreased rate of both uptake and incorporation of 3H-thymidine, but the labeling index remains elevated up to 48 hr. It appears that this is the only exception to the observed good relationship between labeling index and total upt ake of 3H-thymidine. This correlation may be of use in comparing autoradiographic and biochemical data on DNA synthesis in the case of culturing both primary mammary tumors and mammary cancer cell lines. (Author abstract) (22 Refs)

4. STIMULATION OF DNA SYNTHESIS IN MURINE FIBROBLASTS BY THE TUMOUR PROMOTER TELEOCIDIN: RELATIONSHIP TO PHORBOL ESTERS AND VASOPRESSIN.

Collins M, Rozengurt E
Imperial Cancer Res. Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, England
Biochem Biophys Res Commun; 104(4):1159-1166 1982

The influence of the tumor promoter teleocidin (TL) on initiation of DNA synthesis was studied in quiescent Swiss 3T3 and other cells. As determined with the use of (3H)thymidine, TL at 20 nanoM (nM) markedly enhanced DNA synthesis at suboptimal concentrations of serum in Swiss 3T3 cells. In the presence of 2.5% serum, which does not support growth of the 3T3 cells, TL (100 nM) stimulates confluent cells to form multiple layers after 4 days incubation. In serum-free medium, 20 nM TL stimulated DNA synthesis when added in combination with retinoic acid, epidermal growth factor, cholera toxin, platelet-derived growth factor, insulin, fibroblast-derived growth factor (FDGF), and phorbol-12,13-dibutyrate (PDBu). Synergism was shown with FDGF when both TL and retinoic acid were present. No synergism between PDBu and TL was shown in any of the cell types used. Several lines of evidence indicate that TL and phorbol esters bind to the same receptor, or their actions could converge at a post-receptor locus. Thus, prolonged treatment of Swiss 3T3 cells with PDBu leads to a marked decrease in (3H)-PDBu binding sites and marked reduction in subsequent mitogenic response not only to phorbol esters, but also to TL. TL is not synergistic with vasopressin, suggesting that it utilizes the same mitogenic pathway as this physiological ligand. (17 Refs)

5. CA-CALMODULIN MEDIATES THE DNA-SYNTHETIC RESPONSE OF CALCIUM-DEPRIVED LIVER CELLS TO THE TUMOR PROMOTER TPA.

Jones A, Boynton AL, Macmanus JP, Whitfield JF
(c/o Boynton) Animal and Cell Physiology Group, Div. Biological Sciences, Natl. Res. Council Canada, Ottawa, Ontario K1A 0R6, Canada
Exp Cell Res; 138(1):87-93 1982

Incubation in low (0.02 mM)-calcium medium inhibited the proliferation of T51B rat liver cells. Addition of TPA (12-O-tetradecanoyl-phorbol-13-acetate; 50 ng/ml) or calcium (1.25 mM) caused the arrested, calcium-deprived cells to initiate DNA synthesis within 2 hr and eventually to resume proliferating. The rapid DNA-synthetic response to TPA addition appeared to depend on the small amount of calcium remaining in the calcium-deficient medium, because it was blocked by correspondingly small amounts of the specific calcium chelator EGTA or La+++, which displaces calcium from the cell surface and inhibi ts transmembrane calcium fluxes. The common mediation of TPA and calcium actions through Ca-calmodulin complexes was indicated by the following observations: The DNA-synthetic responses to both agents were blocked by the supposedly specific Ca-calmodulin blocker trifluoperazine; the trifluoperazine-induced block was overcome by pure calmodulin; and the responses to both TPA and calcium were blocked by anti-calmodulin antibodies. (Author abstract) (35 Refs)

6. EFFECT OF MAGNESIUM CONTENT ON DENSITY-DEPENDENT REGULATION OF THE ONSET OF DNA SYNTHESIS IN TRANSFORMED 3T3 CELLS.

Rubin H
Dept. Molecular Biology, Univ. California, Berkeley, CA, 94720
Cancer Res; 42(5):1761-1768 1982

A spontaneously transformed clone of BALB/c 3T3 cells became more transformed after more than 90 passages as indicated by increased rounding of cells, multiplication to a higher saturation density, and increased ability to form colonies when suspended in agar. When the extracellular concentration of Mg++ was sharply reduced, the highly transformed cells flattened, assumed the shape of nontransformed cells, and became regularly arranged in cohesive arrays. If crowded when deprived of Mg++, they lost more intracellular Mg++ than did nontransformed and early passage-transformed cells and remained at constant cell density for at least 10 days. The intracellular content of neither Na+ nor K+ changed consistently, with Mg++ deprivation, but the Ca++ content increased more than two-fold. The sensitivity of the onset of DNA synthesis to inhibition by Mg++ deprivation increased with the extent of crowding of the cultures. This was demonstrated by varying population density within a single culture dish as well as from culture to culture. The loss of intracellular Mg++ in low concentrations of extracellular Mg++ increased with cell crowding as did the inhibition of DNA synthesis per fractional loss of intracellular Mg++. Neither deprivation of K+ or Ca++ nor addition of cyclic adenosine 3':5'-monophosphate produced a density-dependent inhibition of DNA synthesis. The results indicate that a reduction of the Mg++ content of highly transformed cells restores density-dependent inhibition of the onset of DNA synthesis, which is a characteristic property of nontransformed cells. The differences in Mg++ retentiveness with population density may reflect differences of intracellular distribution and binding of Mg+, which could in turn explain some of the regulatory effects of population density on metabolism and growth. (Author abstract) (16 Refs)

7. INHIBITION OF DNA SYNTHESIS ACCOMPANIED BY STIMULATION OF PROTEIN SYNTHESIS IN NOVOBIOCIN-TREATED DEVELOPING SEA URCHINS.

Speck WT, Rosenkranz PG, Rosenkranz HS
Dept. Pediatrics, Sch. Medicine, Case Western Reserve, Cleveland, OH, 44106
Mutat Res; 104(1-3):125-130 1982

The effects of novobiocin (NB) on DNA synthesis and protein synthesis were studied in developing American sea urchins, Arbacia punctulata. Gametes of the sea urchin were obtained by electric stimulation and exposed to NB in sea water 10 min after fertilization. Development was examined at intervals. In metabolic studies embryos were exposed to [3H]lysine, [3H]thymidine, or [3H]cytidine after exposure to NB. NB produced developmental abnormalities in a dose-dependent manner. At the highest level tested (1,000 ug NB/ml) embryotoxic effects were characterized by early developmental arrest and abnormal cleavage patterns. At concentrations of 100 or 330 ug NB/ml development was arrested in the blastula stage. At 100 ug/ml blastulae remained viable for 2 days or longer. At concentrations of 10 or 33 ug/ml developmental retardation began in the gastrula stage. At 33 ug ml development was arrested in the pluteus stage. At 10 ug/ml development eventually caught up with growth in controls. Concentrations of 3.3 ug/ml produced no apparent effects on development. DNA synthesis was inhibited by increasing levels of NB. RNA synthesis was inhibited at low levels, but inhibition decreased as NB levels increased. Protein synthesis was stimulated by increasing levels of NB. Hydroxyurea (HU) inhibited DNA synthesis but did not stimulate protein synthesis, indicating that NB and HU act on different enzyme subunits. (17 Refs)

8. CC-1065 (NSC 298223), A NOVEL ANTITUMOR AGENT THAT INTERACTS STRONGLY WITH DOUBLE-STRANDED DNA.

Li LH, Swenson DH, Schpok SL, Kuentzel SL, Dayton BD, Krueger WC
Res. Labs., Upjohn company, Kalamazoo, MI, 49001
Cancer Res; 42(3):999-1004 1982

CC-1065, a novel antibiotic produced by Streptomyces zelensis, was active against several experimental tumors in vivo and a broad spectrum of human tumor cells in vitro. This report describes its biological and biochemical effects on L1210 leukemia cells. CC-1065 is one of the most cytotoxic agents known. The concentrations required for a 50 and 90% inhibition of cell growth are 0.02 and 0.05 ng/ml, respectively. It is about 400 times more cytotoxic than was Adriamycin. The action of CC-1065 is rapid and is dose and time dependent. CC-1065 inhibits DNA synthesis much more than it inhibits RNA and protein synthesis. The concentrations required for a 50% inhibition of DNA synthesis and RNA synthesis are 4 to 6 and 45 to 60 ng/ml, respectively. Although the drug inhibition of DNA synthesis cannot completely account for its cytotoxic effects on L1210 cells, these results, along with those generated by other investigators, suggest that the inhibition of DNA synthesis represents a major mode of action of CC-1065. CC-1065 inhibited both thymidine kinase and DNA polymerase alpha activities, but the effect on highly purified DNA polymerase alpha was more pronounced. At 1 ug/ml, CC-1065 inhibited more than 70% of the enzyme activity. In order to elucidate the mechanism of inhibition of DNA polymerase alpha, the interaction between CC-1065 and DNA was investigated. The studies with thermal melting of DNA and difference circular dichroism measurement indicate that CC-1065 is one of the strongest DNA-binding agents. It induced an increase in thermal melting temperature of calf thymus DNA by at least 31 C. The circular dichroism studies also reveal that CC-1065 binds only to double-stranded DNA but not to heat-denatured DNA or yeast RNA. These observations were supported by those obtained with two other experimental approaches. CC-1065 also appeared to interact with proteins, but the interaction was weak and reversible. (Author abstract) (13 Refs)

9. MODULATION OF 9-BETA-D-ARABINOFURANOSYLADENINE 5'-TRIPHOSPHATE AND DEOXYADENOSINE TRIPHOSPHATE IN LEUKEMIC CELLS BY 2'-DEOXYCOFORMYCIN DURING THERAPY WITH 9-ARABINOFURANOSYLADENINE.

Plunkett W, Benjamin RS, Keating MJ, Freireich EJ
Dept. Developmental Therapeutics, Univ. Texas System Cancer System, M.D. Anderson Hosp. and Tumor Inst., Houston, TX, 77030
Cancer Res; 42(5):2092-2096 1982

The effect of the adenosine deaminase inhibitor, 2'-deoxycoformycin, on cellular nucleotides during therapy with continuous infusion of 9-beta-D-arabinofuranosyladenine (ara-A) has been investigated. In three courses of treatment using increasing doses, the active 5'-triphosphate of ara-A, 9-beta-arabinofuranosyladenine 5'-triphosphate (ara-ATP), accumulated in leukemic cells and erythrocytes from a patient treated for acute lymphocytic leukemia in proportion to the dose of ara-A. The cellular ara-ATP concentration increased more than five-

fold after the injection of a single, nontoxic, but pharmacologically active dose of 2'-deoxycoformycin 24 hr after initiation of ara-A infusion. However, this response was associated with a concomitant increase in the cellular deoxyadenosine triphosphate concentrations to levels equal to or greater than those of ara-ATP throughout the three treatment courses studied. Consistent with previous results using cell-free systems, it was demonstrated that a competitive relationship exists between deoxyadenosine triphosphate and ara-ATP for the inhibition of DNA synthesis in cultured human lymphoblastoid cells and that the ratio of the cellular concentrations of these nucleotides could predict the extent of inhibition of DNA synthesis. Application of this rationale to the nucleotides in the leukemic cells of the patient suggested that administration of 2'-deoxycoformycin may create a cellular biochemical milieu that could be antagonistic to the inhibition of DNA synthesis by ara-ATP. (Author abstract) (32 Refs)

10. THE STRUCTURE OF METHYLATED XANTHINES IN RELATION TO THEIR EFFECTS ON DNA SYNTHE SIS AND CELL LETHALITY IN NITROGEN MUSTARD-TREATED CELLS.

Murnane JP, Byfield JE, Chen CT, Wang CH
Lab. Radiobiology, Univ. California, San Francisco, CA, 94143
Biophys J; 35(3):665-676 1981

The relationship between structural differences in caffeine side chains and differences in the effects of substituted xanthines on cellular processes was studied using the human colon carcinoma cell line HT-29. The effects of caffeine, theobromine, theophylline, paraxanthine, 3-isobutyl-1-methylxanthine, pentoxyphylline, 1-n-butyl-3,7-dimethyl xanthine, 1-n-propyl-3,7-dimethyl xanthine, with or without nitrogen mustard, on HT-29 cells in colony formation assays and on (3H)thymidine incorporation were analyzed in relation to side chain differences. The compounds varied greatly in their effects on HT-29 cells in both tests, with or without HN2. Alkylated xanthines in general produced elevations in DNA synthesis in HN2-damaged DNA at concentrations corresponding to those required for synergistic lethality. Such a result is consistent with the hypothesis that inhibition of replicon initiation in response to HN2, a survival mechanism to prevent expression of damaged genes, is reversed by the xanthines. Enhancement of HN2 lethality is less at toxic levels of xanthines, probably because toxic concentrations prevent cells from passing through the S phase. Lethality of xanthines alone in undamaged cells is apparently related to an immediate inhibition of incorporation of exogenous thymidine. (38 Refs)

11. EFFECTS OF HYDROXYUREA AND BENZO(A)PYRENE ON DNA SYNTHESIS IN THE ISOLATED PERFUSED RAT LUNG.

Imamura T, Busby WF, Goldman ME, Wogan GN, Brunengraber H
Dept. Entomology, Univ. California, Riverside, CA, 92521
Bull Environ Contam Toxicol; 28(2):135-140 1982

An isolated perfused rat lung model was used to determine DNA synthesis in the lung tissue, response of DNA synthesis to hydroxyurea (HU), and effect of in vivo pretreatment with benzo(a)pyrene (BP) on DNA synthesis in the rat lung. DNA synthesis was determined in perfused rat lung by incorporation of (3H)thymidine into lung DNA isolated by neutral density gradient centrifugation. An av of 0.75 mg DNA was obtained from approx 750 mg of lung tissue. (3H)Thymidine incorporation was reduced by over 95% by treatment with 10 milliM HU. Lactate/pyruvate ratios were within the normal physiological range at all HU concentrations tested. Perfusion of rat lungs with 5-50 milliM HU rapidly inhibited lung DNA synthesis with values ranging from 94-97%. With the use of lungs from rats that had been pretreated with BP (6 mg/kg in corn oil, ip for 3 days), DNA synthesis was inhibited by 60%, although there was no indication of lung damage. DNA synthesis was inhibited by 90-95% with HU both in lungs from BP-treated rats and lungs from corn oil onl y controls; there was no significant difference between the two groups. (11 Refs)

12. THE EFFECT OF BENZO(A)PYRENE ON DNA SYNTHESIS AND DNA POLYMERASE ACTIVITY OF RAT LIVER MITOCHONDRIA.

Salazar I, Tarrago-Litvak L, Gil L, Litvak S
Departamento de Bioquimica, Facultad de Medicina, Universidad de Chile, Casilla 6671, Santiago 7, Chile
FEBS Lett; 138(1):45-49 1982

DNA synthesis in liver mitochondria of benzo(a)pyrene (BP: 40 mg/kg)-treated rats was higher than in mitochondria from untreated rats, both at different protein concentrations and over different incubation times, but the solubilized mitochondrial DNA polymerase activity was higher in the control preparation. Dideoxythymidine triphosphate (ddTTP) inhibited DNA synthesis in whole mitochondrial preparations, with a much more profound effect in mitochondria from untreated rats. Detergent treatment showed that the difference between treated and untreated mitochondria was not due to BP effect on organelle membrane permeability. Ethidium bromide produced a polymerase inhibiting effect similar to that of ddTTP, but aphidicolin did not. (21 Refs)

13. THE HELA DNA-SYNTHESIS INHIBITION TEST AS A RAPID SCREEN FOR MUTAGENIC CARCINOGENS.

Painter RB, Howard R
Lab. Radiobiology, Univ. California, San Francisco, CA, 94143
Mutat Res; 92(1/2):427-437 1982

DNA synthesis inhibition (DSI) in HeLa and other mammalian cells was used for rapid detection of relatively strong mutagenic carcinogens. Of 90 agents tested for DSI in HeLa cells, the weaker DNA-damaging agents generally gave positive results only at high doses (1 milliM or greater) and very weak agents (eg, saccharin) gave negative results. One false positive result (cytochalasin B) was observed, with an atypical dose-response curve. The DSI test seems unsuitable for complex mixtures, probably because the DSI activity of a DNA-damaging agent may be masked by the actions of agents without DNA-damaging activity but which inhibit DNA synthesis by other means. (27 Refs)

14. CRITICAL APPRAISAL OF THE 'MOUSE TESTICULAR DNA-SYNTHESIS INHIBITION TEST' FOR THE DETECTION OF MUTAGENS AND CARCINOGENS.

Donatsch P, Gurtler J, Matter BE
Preclinical Res. Dept., Toxicology Unit, Sandoz Ltd., Basel, Switzerland
Mutat Res; 92(1/2):265-273 1982

The inhibition of testicular DNA synthesis (DSI test) in mice, proposed as a short-term in vivo assay for potential environmental mutagens and carcinogens, is examined. Male CD-1 mice were given single large doses of 1 of 10 chemicals whose mutagenic activities had been identified by other methods. The effects of reduced testicular temperature (caused by immobilization in a water bath at low temperatures) on testicular DNA synthesis were also studied. Hypothermia was observed after

treatment with 6/6 nonmutagens and 3/4 known mutagens, and was associated with inhibition of testicular DNA synthesis. Testicular DNA synthesis inhibition caused by some of the test agents could be reduced under isothermal conditions. The DSI test is not thought to be a reliable means for detecting potential environmental mutagens and carcinogens. (6 Refs)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

15. CHANGES IN DNA POLYMERASES ALPHA, BETA, AND GAMMA DURING THE REPLICATIVE LIFE SPAN OF CULTURED HUMAN FIBROBLASTS.

Krauss SW, Linn S
Dept. Biochemistry, Univ. California, Berkeley, CA, 94720
Biochemistry; 21(5):1002-1009 1982

DNA polymerases from IMR-90 human diploid fibroblasts at various passage levels and from HeLa cells were purified and fractionated into alpha1, alpha2, alpha3, beta, and gamma species and subspecies, and then the accuracy with which each one copied synthetic template-primers was measured in the presence of Mn++ and Mg++. All activities from fibroblasts of later population doubling levels incorporated noncomplementary triphosphates more frequently than did the same polymerase type from earlier population doubling levels. HeLa polymerase activities copied several different templates in the presence of Mn++ with greater fidelity than enzymes from fibroblasts of population doubling level 27 or greater. The total DNA polymerase activity extracted from IMR-90 cells decreased with increasing population doubling levels. The alpha-polymerase activity declined with increasing population doubling levels, while beta-polymerase activity remained relatively constant, except at the very end of the cellular replicative life span. In addition, the amounts of alpha2 and alpha3 became progressively lower relative to alpha1, and a new alpha-type polymerase activity, alpha0, appeared upon diethylaminoethylcellulose chromatography. HeLa cells also contained three alpha species, though two of them eluted from diethylaminoethylcellulose at higher phosphate concentrations than alpha species from fibroblasts. Postconfluent IMR-90 cells of population doubling level 21 had a decreased level of alpha-polymerase relative to that recovered from rapidly growing cells. This polymerase activity had some chromatographic properties similar to enzyme from late-passage cells. In addition, the alpha-, beta-, and gamma-polymerases from these cells had decreased fidelities relative to those isolated from subconfluent cells. (Author abstract) (34 Refs)

16. DNA-PROCESSING ACTIVITIES ASSOCIATED WITH THE PURIFIED ALPHA, BETA2, AND ALPHABETA5AB MOLECULAR FORMS OF AVIAN SARCOMA VIRUS RNA-DEPENDENT DNA POLYMERASE.

Hizi A, Gazit A, Guthmann D, Yaniv A
Dept. Cell Biology and Histology, Sackler Sch. Medicine, Tel-Aviv Univ., Tel-Aviv, Israel
J Virol; 41(3):974-981 1982

The RNA-dependent DNA polymerase purified from B77 avian sarcoma virus exhibited two distinct DNA-processing activities. The alpha and beta2 isoenzymes possessed an endodeoxyribonuclease activity capable of nicking simian virus 40 superhelical DNA, whereas the alphabeta isoenzyme performed as an untwisting topoisomerase. Both activities associated with the three molecular forms of the retroviral DNA polymerase were dependent on the presence of either Mn++ or Mg++ ions. From analysis of the denatured DNA products, it is apparent that the alpha and beta2 isoenzymes introduced two nicks, one per each strand in the superhelical simian virus 40 DNA molecules, whereas the alphabeta polymerase converted these supercoiled molecules to the relaxed covalently closed circular form. The notion that the DNA-processing activities are located on the DNA polymerase molecules was supported by the following: (i) the three isoenzymes were of a high purity; (ii) the activities cosedimented in glycerol gradients with the DNA polymerase activities of the alpha, beta2, and alphabeta molecular forms; and (iii) immunoglobulin directed against the purified polymerase immunoprecipitated the DNA-processing activities. Chemical treatments of the DNA polymerase molecules (with pyridoxalphosphate, iodoacetamide, and sulfhydryl reagents), which inhibited the polymerase activity, also suppressed the endonucleolytic and topisomerase activities, suggesting that cysteine and amino groups play an important role in the active sites of the DNA-processing activities as well. (Author abstract) (22 Refs)

17. POSSIBLE INVOLVEMENT OF DNA POLYMERASES ALPHA AND BETA IN BLEOMYCIN-INDUCED UNSCHEDULED DNA SYNTHESIS IN PERMEABLE HELA CELLS.

Seki S, Ohashi M, Ogura H, Oda T
Dept. Biochemistry, Cancer Inst., Okayama Univ. Medical Sch., Okayama 700, Japan
Biochem Biophys Res Commun; 104(4):1502-1508 1982

Possible difference in repair polymerases in bleomycin (BLM)-induced unscheduled DNA synthesis (USD) in some permeabilized human and rodent cells was studied with the use of selective inhibitors for DNA polymerases. As to the inhibitors used, aphidicolin and N-ethylmaleimide, had greater effect on polymerase alpha (P-A) while 2',3'-dideoxythymidine-5'-triphosphate had greater effect on polymerase beta (P-B). Results suggest that both polymerases are involved in BLM-induced USD in HeLa-S3 cells and probably in other human cells such such as HEp-2, KB and WI-38 VA-13. In the rodent cells tested (SR-C3h/He, Balb/c 3T3, 3Yl, and XC), DNA P-B was mainly responsible for BLM-induced USD, although minor roles for DNA P-A could not be ruled out. (30 Refs)

18. SPECIFIC SEQUENCES IN NATIVE DNA THAT ARREST SYNTHESIS BY DNA POLYMERASE ALPHA.

Weaver DT, DePamphilis ML
Dept. Biological Chemistry, Harvard Medical Sch., Boston, MA, 02115
J Biol Chem; 257(4):2075-2086 1982

The effect of the DNA sequence of a template on the progress of DNA polymerase a lpha was determined at the resolution of single nucleotides by using single-stranded, circular phiX174 DNA as a template and unique phiX174 DNA fragments terminally labeled at their 3'-ends as primers. Therefore, the amount of radioactivity observed was always proportional to the number and not the length of nascent DNA chains. Extension of a primer by alpha-polymerase revealed that 3'-ends of nascent DNA chains accumulated at specific arrest sites consisting of GC-rich sequences of 1-8 bases distributed nonuniformly along the template with intervening sequences of 0-140 bases. The precise location and composition of these sites were

determined by DNA sequencing methods, but a consensus sequence was not detected. However, the same pattern of arrest sites recognized by DNA polymerase alpha from CV-1 cells also arrested alpha-polymerase from HeLa cells, calf thymus tissue, and phage T4 DNA polymerase. An exception was DNA polymerase I from Escherichia coli which was insensitive to arrest signals. Analysis of recombinant DNA templates containing phiX174 DNA inserted into M13 DNA revealed that arrest sites were defined exclusively by those sequences within 24 bases upstream and 140 bases downstream of the arrest point; long range interactions between template sequences were not involved. One of the strongest arrest sites was located in the first two nucleotides at the base of the stem on the primer-proximal side of a stable hairpin structure, suggesting that such structures arrest the polymerase. However, only 28% of all arrest site nucleotides were found in this position; the rest were up to 25 bases upstream from any computer-predicted hairpin. Therefore, all arrest sites cannot be defined by secondary structure alone, although proximity to secondary structures may amplify normal variations in the rate of DNA elongation caused by primary sequences. (Author abstract) (48 Refs)

19. PROBABLE INVOLVEMENT OF A GLYCOCONJUGATE IN IMR-32 DNA SYNTHESIS: DECREASE OF DNA POLYMERASE ALPHA2 ACTIVITY AFTER TUNICAMYCIN TREATMENT.

Bhattacharya P, Basu S
(c/o Basu) Dept. Chemistry, Biochemistry, Biophysics Program, Univ. Notre Dame, Notre Dame, IN, 46556
Proc Natl Acad Sci USA; 79(5):1488-1491 1982

Multiple forms of DNA polymerase alpha activity (alpha1, alpha2, and alpha3) from human neuroblastoma IMR-32 cells untreated or treated with tunicamycin (3 ug/ml) were separated by DEAE-cellulose column chromatography. Loss of 40-60% of DNA polymerase alpha2 activity was observed in tunicamycin-treated cells. Ricin 1B, a subunit of intact ricin (Mr, 64,000), was found to be a specific inhibitor of DNA polymerase alpha2 isolated from control IMR-32 cells. However, DNA polymerase alpha2 isolated from tunicamycin-treated cells was insensitive to ricin 1B. Heat treatment studies at 50 C showed two completely different inactivation profiles for the DNA polymerase alpha2 enzymes isolated from the tunicamycin-treated and untreated cells. A probable involvement of a beta-linked galactose-containing carbohydrate chain in the catalytic subunit of DNA polymerase alpha2 is suggested. (Author abstract) (28 Refs)

20. COMPARATIVE EFFECTS OF THE 5'-TRIPHOSPHATES OF 9-BETA-(2'-AZIDO-2'-DEOXY-D-ARABINOFURANOSYL)ADENINE AND 9-BETA-D-ARABINOFURANOSYLADENINE ON DNA POLYMERASES FROM L1210 LEUKEMIA CELLS.

Allaudeen HS, Kozarich JW, Sartorelli AC
Dept. Pharmacology and Developmental Therapeutics Program, Comprehensive Cancer Center, Yale Univ. Sch. Medicine, New Haven, CT, 06510
Nucleic Acids Res; 10(4):1379-1387 1982

The compound 9-beta-(2'-Azido-2'-deoxy-D-arabinofuranosyl)adenine (arazide) causes greater and significantly more persistent inhibition of [3H]thymidine incorporation into the DNA of neoplastic cells than the related agent 9-beta-D-arabinofuranosyladenine (araA). To elucidate the mechanism(s) responsible, we compared the effects of arazide and araA 5'-triphosphates on DNA polymerases alpha and beta of L1210 leukemia cells. Both nucleoside triphosphate analogs inhibited DNA polymerase alpha activity by competing with dATP; only araATP was inhibitory to DNA polymerase beta. Arazide triphosphate was at least four times more active than araATP as an inhibitor of DNA polymerase alpha. Preincubation of DNA polymerase alpha with either agent did not result in enzyme inactivation. The results suggest that interference with DNA polymerase alpha activity by arazide triphosphate may be in part responsible for the inhibition of DNA synthesis produced by arazide in neoplastic cells. (Author abstract) (22 Refs)

21. DNA POLYMERASE REQUIREMENTS FOR PARVOVIRUS H-1 DNA REPLICATION IN VITRO.

Kollek R, Tseng BY, Goulian M
Institut fur Biochemie, Univ. Munchen, Karlstrasse 23, D-8000 Munchen 2, W. Germany
J Virol; 41(3):982-989 1982

An in vitro system using nuclei from parvovirus H-1-infected cells was used to characterize the influence of inhibitors of mammalian DNA polymerases on viral DNA synthesis. The experiments tested the effects of aphidicolin, which is highly specific for DNA polymerase alpha, and 2',3'-dideoxythymidine-5'-triphosphate (ddTTP), which inhibits cellular DNA polymerase in the order gamma greater than beta greater than alpha. Both aphidicolin and ddTTP were inhibitory, indicating that both polymerase alpha and a ddTTP-sensitive enzyme are required for viral DNA synthesis. This was seen more clearly in kinetic measurements, which indicated an initial period of rapid DNA synthesis with the participation of polymerase alpha, followed by a period of less rapid, but more sustained, rate of DNA synthesis carried out by a ddTTP-sensitive enzyme, probably polymerase gamma. One interpretation of the results is that polymerase alpha fun ction in a strand displacement stage of the viral DNA replication mechanism, whereas polymerase gamma serves to convert the displaced single strands back to double-strand replicative form. (Author abstract) (42 Refs)

22. PYROPHOSPHATE ANALOGUES AS INHIBITORS OF DNA POLYMERASES OF CYTOMEGALOVIRUS, HERPES SIMPLEX VIRUS AND CELLULAR ORIGIN.

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Biochim Biophys Acta; 696(2):115-123 1982

Several pyrophosphate analogues have been compared for their ability to inhibit the activities of isolated cytomegalovirus (CMV) DNA polymerase, herpes simplex virus Type 1 (HSV 1) DNA polymerase and calf thymus DNA polymerase alpha. The most effective inhibitors were phosphonoformate and phosphonoacetate. Although not identical, the structural requirements for compounds inhibitory to CMV and HSV-1 DNA polymerase were specific, with two negatively charged groups in close vicinity. The CMV DNA polymerase was more susceptible to certain phosphonoacetates containing bulky hydrophobic alpha-substituents than was the HSV-1 DNA polymerase. No example of the converse preference was found. The inhibition of CMV DNA polymerase by phosphonoformate, hypophosphate, alpha-hydroxyphosphonoacetate and alpha-nonylphosphonoacetate was linear non-competitive with the deoxyribonucleoside triphosphates as variable substrates. Phosphonoformate, phosphonoacetate, and to a lesser extent alpha-hydroxyphosphonoacetate, carbonyldiphosphonate and

alpha-nonylphosphonoacetate also inhibited the focus formation by CMV in cell-culture. (Author abstract) (33 Refs)

23. INHIBITORY EFFECTS OF VARIOUS 5-HALOGENATED DERIVATIVES OF 1-BETA-D-ARABINOFURANOSYLURACIL 5'-TRIPHOSPHATE ON DNA POLYMERASES FROM MURINE CELLS AND ONCORNAVIRUS: SUBSTITUENT EFFECTS ON INHIBITORY ACTION.

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Biochemistry; 21(5):1019-1024 1982

The effects of four 5-halogenated derivatives of 1-beta-D-arabinofuranosyluracil 5'-triphosphate (aUTP) on the activity of DNA polymerases from mouse cells and oncornavirus were examined in comparison with that of the mother compound, aUTP, and also with those of the sister compounds, 5-alkylated derivatives of aUTP. The newly synthesized and tested compounds in this study were 1-beta-D-arabinofuranosyl-5-fluorouracil 5'-triphosphate (aFUTP), 1-beta-D-arabinofuranosyl-5-chlorouracil 5'-triphosphate (aClUTP), 1-beta-D-arabinofuranosyl-5-bromouracil 5'-triphosphate (aBrUTP), and 1-beta-D-arabinofuranosyl-5-iodouracil 5'-triphosphate (aIUTP). The results are summarized. Relationships between the chemical structures of the analogues and their inhibitory effects are discussed according to inductive, hyd rophobic, and steric characteristics of the substituents on the 5 position of the uracil nucleus. It was concluded that both steric and hydrophobic effects of the halogen substituents were important for the inhibition of all DNA polymerases and that the inductive effect of halogen atoms was clearly observed for DNA polymerase beta with activated DNA and RLV DNA polymerase with (rA)n.(dT)12-18. (22 Refs)

24. QUANTITATION OF RNA-DEPENDENT PLATELET DNA POLYMERASE IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS.

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Br J Haematol; 50(3):521-530 1982

Platelets from 28 patients with the myeloproliferative diseases (MPD) polycythemia vera (9), essential thrombocythemia (6), myelofibrosis with myeloid metaplasia (5) and chronic myelogenous leukemia (8) were examined for an RNA-dependent DNA polymerase activity using standardized conditions permitting highly reproducible quantitation. Low levels of activity were detected in platelets of normal individuals, but platelets of nearly all MPD patients (25/28) possessed higher levels. The polymerase activity correlated with diagnosis ($p = 0.001$) and did not correlate with platelet counts (p greater than 0.2). Quantitation of this RNA-dependent DNA polymerase activity may be a useful parameter in the diagnosis of myeloproliferative disorders. (Author abstract) (20 Refs)

25. REVERSE TRANSCRIPTASE ASSOCIATED WITH AVIAN SARCOMA-LEUKOSIS VIRUSES. I. COMPARISON OF INTRA-VIRION CONTENT OF MULTIPLE ENZYME FORMS.

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J Biochem (Tokyo); 91(1):311-322 1982

The RNA-dependent DNA polymerase (the reverse transcriptase) was solubilized from three related strains of avian sarcoma virus (ASV B77, ASV tsLA334, and ASV QV2) as well as avian myeloblastosis virus (AMV) and a chicken endogenous virus (RAV-0), by a combination of non-ionic detergent treatment and CsCl step-gradient centrifugation, and was subsequently separated into individual enzyme forms by poly(C)-agarose column chromatography. The newly developed two-step method allowed us to purify the three molecular forms (alpha, alphabeta-, and beta-form) of highly active enzyme rapidly and quantitatively from all the five virus strains examined. The molar ratio of the three enzyme forms differed among the virus strains: for the three sarcoma viruses, the major species was the alphabeta-form enzyme, the putative holoenzyme, and the alpha- and beta-form enzymes were less than a few percent and 15-25%, respectively, while the alpha-form enzyme content was higher for the two leukosis viruses than for the three sarcoma viruses. Both the total DNA polymerase activity and the content of the two enzyme subunits in purified virions of the three sarcoma virus was in the following order: ASV tsLA334 greater than ASV B77 greater than ASV QV2, which paralleled the virus yield at a permissive temperature in roller bottle cultures of chick embryo fibroblasts. No alteration was found in the thermolability of DNA polymerases between tsLA334, which carries ts mutations affecting both virus growth and cell-transformation, and other viruses. (Author abstract) (30 Refs)

26. REVERSE TRANSCRIPTASE ASSOCIATED WITH AVIAN SARCOMA-LEUKOSIS VIRUSES. II. COMPARISON OF SUBUNIT STRUCTURE AND CATALYTIC PROPERTIES.

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J Biochem (Tokyo); 91(1):323-330 1982

The three enzyme forms (alpha, alphabeta-, and beta-form) of the RNA-dependent DNA polymerase (the reverse transcriptase) from three strains of avian sarcoma virus (ASV B77, ASV tsLA334, and ASV QV2) and one exogenous (avian myeloblastosis virus (AMV)) and one endogenous avian leukosis virus (Rous-associated virus type-0 (RAV-0)) were compared with each other in subunit structure and catalytic properties. Despite the gross similarity in the subunit mol wt (Mr(alpha) = 65,000 and Mr(beta) = 92,000), minor differences were found in the molecular size of the subunit as determined by SDS-gel electrophoresis, the order being ASV tsLA334 less than or equal to ASV QV2 less than ASV B77 less than or equal to RAV-O = AMV. The structural differences were supported by analysis of peptide fragments after treatment with S aureus V8 protease. Although the general catalytic properties of the purified enzymes from the five virus strains were similar, the selectivity of template-primer differed in the RAV-O enzymes. The template-primer selectivity of .he reverse transcriptases from all five virus strains tested was also found to be greatly influenced by the reaction temperature for DNA synthesis, resulting in a temperature-dependent increase of

poly(dG) synthesis over [(A)n].[(dT)12-18]-dependent poly(dT) synthesis. The RAV-O enzymes required a lower temperature for DNA synthesis, particularly for [(C)n].[(dG)12-18]-dependent poly(dG) synthesis. (Author abstract) (28 Refs)

27. INHIBITION OF DNA METHYLTRANSFERASE AND INDUCTION OF FRIEND ERYTHROLEUKEMIA CELL DIFFERENTIATION BY 5-AZACYTIDINE AND 5-AZA-2'-DEOXYCYTIDINE.

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J Biol Chem; 257(4):2041-2048 1982

Treatment of Friend erythroleukemia cells with the antileukemic drugs 5-azacytidine and 5-aza-2'-deoxycytidine leads to rapid, time-dependent, and dose-dependent decrease of DNA methyltransferase activity and synthesis of markedly undermethylated DNA. Since this DNA is at lea st partially methylated in vivo and serves as an excellent substrate for methylation in vitro, hypomethylation of DNA in analog-treated cells appears to result from the loss of DNA methyltransferase, rather than from an inherent inability of 5-azacytosine-substituted DNA to serve as a methyl acceptor. Inhibition of DNA synthesis blocks the loss of DNA methyltransferase activity while inhibitors of RNA synthesis do not, suggesting that the analogs must be incorporated into DNA to mediate their effect on the enzyme, and that minor substitution of 5-azacytosine for cytosine in DNA (approx 0.3%) suffices to inactivate more than 95% of the enzyme in the cell. Several lines of evidence link changes in the pattern of DNA modification with differentiation. In this regard, it is significant that 5-azacytidine and 5-aza-2'-deoxycytidine act as weak inducers of erythroid differentiation of Friend erythroleukemia cells in the same concentration range where they affect DNA methyltransferase activity. For differentiation to proceed, the cells must be washed free of the drugs. Less than 24 hr later, normal levels of DNA methyltransferase activity are restored and within 48 hr, DNA isolated from cells is not detectably undermethylated. This may in part explain why 5-azacytidine and 5-aza-2'-deoxycytidine induce differentiation in less than 15% of the population despite their initial profound effect on DNA methylation. (Author abstract) (35 Refs)

28. REGULATION OF THYMIDINE KINASE ENZYME LEVEL IN SERUM-STIMULATED MOUSE 3T6 FIBROBLASTS.

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Exp Cell Res; 138(1):79-85 1982

This report describes the regulation of thymidine kinase (TK) enzyme level in mouse 3T6 fibroblasts experiencing a serum-induced transition between the resting (G0) and growing states. The specific activity of TK is about 200-500 pmoles TMP formed x min-1 x mg protein-1 in exponentially growing cells but is scarcely detectable (usually less than 5 pmoles TMP formed x min-1 x mg protein(-1)) in cells resting at confluence in medium containing 0.5% serum. When the resting cells are serum-stimulated to re-enter the cell cycle, TK activity remains at the resting level until about 12 hr following stimulation, but then increases dramatically as the cells enter S phase. Experiments with inhibitors of protein or RNA synthesis indicate that the increase in enzyme level is the result of de novo synthesis of the enzyme, and that the increase depends on transcription. The increase in TK level is not blocked when cells are stimulated in the presence of inhibitors of DNA synthesis, indicating that there is no tight coupling between these two processes. The increase is not blocked by the presence of thymidine ranging in concentration from 10(-7) to 10(-3) M, suggesting that fluctuations in the intracellular concentrations of thymidine or thymidylic acid do not affect the increase in TK level. When cells are treated with cycloheximide, the level of TK decreases with a 4-5 hr half-life, indicating that the enzyme is relatively unstable. When the serum stimulus is withdrawn from cells in S phase (20 hr following stimulation), the TK level decreases with the same 4 hr half-life beginning about 6 hr following serum withdrawal. The ability of the cell to control the rate of production of TK over a very wide range, along with the relative instability of the enzyme appear to be responsible for the rapid and wide variations in TK level during the cell cycle. The increase in the level of TK appears to be regulated in a manner similar to that of dihydrofolate reductase and thymidylate synthetase in serum-stimulated 3T6 cells, suggesting that the expression of these genes (and perhaps those for other S phase enzymes) may be coordinated by a common control signal. (Author abstract) (23 Refs)

29. DNA LIGASE AND DNASE ACTIVITIES IN MOUSE ERYTHROLEUKEMIA CELLS DURING DIMETHYL SULFOXIDE-INDUCED DIFFERENTIATION.

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Cancer Res; 42(4):1300-1306 1982

DNA ligase and DNase levels were measured in cell-free extracts from untreated mouse erythroleukemia (MEL) cells and from cells treated with dimethyl sulfoxide (Me2SO) to induce erythroid differentiation. The DNase activity present in the extracts was sensitive to inhibition by G-actin and was, therefore, presumed to be DNase I. When the MEL cells were induced to differentiate by culturing in the presence of 1.8% Me2SO for 3 or 4 days, the apparent activity of DNA ligase decreased to approx 12% of the value in untreated MEL cells. In contrast, the apparent DNase I activity of the extracts from Me2SO-treated cells increased over that in extracts from untreated cells by a factor of 2. The activity of acid phosphatase, a lysosomal enzyme, remained unchanged. When strain DR-10, a mutant of the MEL cells which does not undergo Me2SO-induced differentiation, was treated with Me2SO, the DNA ligase and DNase activities of extracts from these cells remained unchanged as compared to extracts from untreated DR-10 cells. Therefore, the marked decrease in the level of DNA ligase activity appeared to be related to the process of differentiation in the Me2SO-treated MEL cells. (Author abstract) (39 Refs)

MECHANISMS OF DNA REPAIR

30. A REDUCED CAPACITY FOR UNSCHEDULED DNA SYNTHESIS IN LYMPHOCYTES FROM INDIVIDUALS EXPOSED TO PROPYLENE OXIDE AND ETHYLENE OXIDE.

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Mutat Res; 104(1-3):193-200 1982

Inhibition of unscheduled DNA synthesis (UDS) was studied in 23 workers (25-59 yr old, mean age 41 yr) exposed to propylene oxide (PO) as process workers for 1-20 yr (mean 10 yr) and in 5 male workers (25-44 yr old, mean age 32 yr) exposed to ethylene oxide (EO) as sterilizing, packing, or truck-driving personnel for 0.3-5 yr (mean 2.9 yr). Eighteen of 23 PO-exposed workers were smokers. Controls were 12 male workers (21-46 yr old, mean age 30 yr) not exposed occupationally to any mutagenic chemical compound. Five of the controls were smokers. UDS induced by N-acetoxy-2-acetylaminofluorene (NA-AAF) and NA-AAF binding to DNA were the methods used. Use of [3H]NA-AAF enabled the direct assessment of initial NA-AAF damage to DNA, which in turn served as the substrate for induction of UDS, and thereby repair of NA-AAF lesions in the DNA. Lymphocyte samples from both the controls and the PO-exposed workers varied considerably in values for NA-AAF-induced UDS. UDS variation was dependent on the level of NA-AAF bound to DNA after a 30-min incubation of lymphocytes in the presence of 10 uM [3H]NA-AAF. The correlation coefficients between NA-AAF-induced UDS and NA-AAF binding to DNA were highly significant for both the controls and PO-exposed workers. PO-plus EO-exposed workers exhibited significant reductions in NA-AAF-induced UDS when compared to the controls. The level of [3H]NA-AAF covalent binding to DNA was not significantly reduced by PO- and EO-exposure. Results indicate that exposure to relatively low doses of PO and EO can cause individuals to be at increased risk due to a reduced capacity to repair DNA lesions. (12 Refs)

31. ABNORMAL LEVELS OF UV-INDUCED UNSCHEDULED DNA SYNTHESIS IN ATAXIA TELANGIECTASIA CELLS AFTER EXPOSURE TO IONIZING RADIATION.

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Mutat Res; 92(1/2):439-446 1982

The baseline levels of UV-induced unscheduled DNA synthesis (UDS) in normal human fibroblast cell lines (C3RO and C5R0) and ataxia-telangiectasia (AT) fibroblast cell lines (AT5BI, AT4BI, AT2BE, AT1PWA, and AT3BI) were determined to be the same. Exposure to x-rays (XR: up to 20,000 rads) significantly altered UV-induced UDs in the AT cells but not in the normal cells. In AT cell lines showing a reduced capacity for gamma-ray-induced repair DNA synthesis (excision-deficient AT cell lines AT3BI and AT2BE), XR exposure inhibited UV-induced UDS. In AT cell lines showing a normal capacity for gamma-ray-induced repair DNA synthesis (excision-proficient AT cell lines), XR exposure stimulated UV-induced UDS. The excision-deficient and excision-proficient AT cell lines showed different dose-response relationships for both UV and XR. The two types of AT cells may have different molecular defects. Altered levels of repair DNA synthesis after UV exposure in AT cells may be secondary consequences of the way the cells handle DNA damage induced by ionizing radiation. (14 Refs)

32. THE EFFECT OF BLEOMYCIN ON DNA SYNTHESIS IN ATAXIA TELANGIECTASIA LYMPHOID CELLS.

Cohen MM, Simpson SJ
Div. Genetics, Children's Memorial Hosp., 2300 Children's Plaza, Chicago, IL, 60614
Environ Mutagen; 4(1):27-36 1982

The effect of bleomycin (BLM) on DNA synthesis was determined in (methyl-3H)thymidine-labeled lymphoid cells from five patients with ataxia telangiectasia (AT) and three patients with xeroderma pigmentosum (XP) compared to those from four normal subjects. BLM (5, 10, 25 and 50 ug/ml) treatment of AT cells over a period of 4 hr led to some inhibition of incorporation compared to normal cells. DNA synthesis of AT cells determined at hourly intervals after exposure to BLM (1, 5, 10, and 25 ug/ml) showed that AT cells did not attain normal levels during the 24-hr observation period whereas normal cells recovered within 15-18 hr. These differences were not found after exposure of the cells to mitomycin C. Following BLM treatment and preincubation with hydroxyurea, residual DNA synthesis was similar in AT, XP and normal lymphoid cells, suggesting that the capacity to repair the induced DNA lesion is present. (30 Refs)

33. REPAIR AND REPLICATION OF DNA IN HEREDITARY (BILATERAL) RETINOBLASTOMA CELLS AFTER X-IRRADIATION.

Cleaver JE, Char D, Charles WC, Rand N
Lab. Radiobiology and Environmental Health, Univ. California, San Francisco, CA, 94143
Cancer Res; 42(4):1343-1347 1982

Fibroblasts from patients with hereditary retinoblastoma reportedly exhibit increased sensitivity to killing by x-rays. Although some human syndromes with similar or greater hypersensitivity to DNA-damaging agents (eg, x-rays, ultraviolet light, and chemical carcinogens), such as xeroderma pigmentosum, are deficient in DNA repair; most do not have such clearly demonstrable defects in repair. Retinoblastoma cells appear to be normal in repairing single-strand breaks and performing repair replication after x-irradiation and also in synthesizing poly(adenosine diphosphoribose). Semiconservative DNA replication in these cells, however, is slightly more resistant than normal after x-irradiation, suggesting that continued replication of damaged parental DNA could contribute to the pathogenesis of the disease. This effect is small, however, and may be a consequence rather than a cause of the fundamental enzymatic abnormality in retinoblastoma that causes the tumorigenesis. (Author abstract) (42 Refs)

34. XERODERMA PIGMENTOSUM VARIANTS.

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Cytogenet Cell Genet; 31(3):188-192 1981

Xeroderma pigmentosum (XP) consists of the subgroups A, B, C, D, E, F, G, and va riant. The first seven subgroups have a defect in the mechanisms of excision repair of DNA damage by UV radiation. The XP variant subgroup has normal excision repair but abnormal replication of UV-damaged DNA. The defect may be the loss of a gene product required for replication past damaged sites. Cultures of XP variant cells from a variety of ethnic groups and geographical areas are available

and should be useful for investigating actinic carcinogenesis in XP. (26 Refs)

35. INACTIVATION OF ULTRAVIOLET REPAIR IN NORMAL AND XERODERMA PIGMENTOSUM CELLS BY METHYL METHANESULFONATE.

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Lab. Radiobiology and Environmental Health, Univ. California, San Francisco, San Francisco, CA, 94143
Cancer Res; 42(3):860-863 1982

Excision repair of ultraviolet damage in the DNA of normal and xeroderma pigmentosum (Groups C, D and variant) cells was inactivated by exposure of cells to methyl methanesulfonate immediately before irradiation independent of the presence of 0 to 10% fetal calf serum. The inactivation could be represented by a semilog relationship between the amount of repair and methyl methanesulfonate concentration up to approx 5 mM. The inactivation can be considered to occur as the result of alkylation of a large (about 10(6) daltons) repair enzyme complex, and the dose required to reduce repair to 37% for most cell types was between 4 and 7 mM. No consistent, large difference in sensitivity to methyl methanesulfonate was found in any xeroderma pigmentosum complementation group compared to normal cells, implying that reduced repair in these groups may be caused by small inherited changes in the amino acid composition (ie, point mutations or small deletions) rather than by losses of major components of the repair enzyme complex. (Author abstract) (19 Refs)

36. A DEFICIENCY IN THE REPAIR OF UV AND GAMMA-RAY DAMAGED DNA IN FIBROBLASTS FROM COCKAYNE'S SYNDROME.

Rainbow AJ, Howes M
Dept. Radiology, McMaster Univ., Hamilton, Ontario L8S 4J9, Canada
Mutat Res; 93(1):235-247 1982

DNA-repair deficiencies were investigated in fibroblasts from five unrelated patients with Cockayne's syndrome (CS) and two patients heterozygous for CS. Suspensions of adenovirus type 2 were exposed to UV or gamma irradiation. Fibroblasts were then infected with irradiated or nonirradiated virus and then incubated. The fibroblasts were then assayed by immunofluorescence methods for expression of viral structural antigens. Results obtained from CS fibroblasts were compared with results obtained from normal and xeroderma pigmentosum (XP) fibroblasts. Host-cell reactivation (HCR) of viral function for both UV- and gamma-irradiated virus was reduced in homozygous CS fibroblasts. The dose of UV required to reduce viral function in homozygous CS fibroblasts by 37% (D37) was 14-35% of the D37 for normal cells. D37 for gamma-irradiation of CS cells was 61-80% of that for normal cells. One heterozygous CS strain had a D37 for UV-irradiated virus 68% of normal, intermediate between the D37 for homozygous CS and normal cells. The other heterozygous CS strain had a normal D37. Results indicate that CS fibroblasts have a defect in DNA repair mechanisms. (41 Refs)

37. DEFICIENT REPAIR OF CHEMICAL ADDUCTS IN ALPHA DNA OF MONKEY CELLS.

Zolan ME, Cortopassi GA, Smith CA, Hanawalt PC
Dept. Biological Sciences, Stanford Univ., Stanford, CA, 94305-2493
Cell; 28(3):613-619 1982

We have examined excision repair of DNA damage in the highly repeated alpha DNA sequence of cultured African green monkey cells. Irradiation of cells with 254 nm ultraviolet light resulted in the same frequency of pyrimidine dimers in alpha DNA and the bulk of the DNA. The rate and extent of pyrimidine dimer removal, as judged by measurement of repair synthesis, was also similar for alpha DNA and bulk DNA. In cells treated with furocoumarins and long-wave-length ultraviolet light, however, repair synthesis in alpha DNA was only 30% of that in bulk DNA, although it followed the same time course. We found that this reduced repair was not caused by different initial amounts of furocoumarin damage or by different sizes of repair patches, as we found these to be similar in the two DNA species. Direct quantification demonstrated that fewer furocoumarin adducts were removed from alpha DNA than from bulk DNA. In cells treated with another chemical DNA-damaging agent, N-acetoxy-2-acetylaminofluorene, repair synthesis in alpha DNA was 60% of that in bulk DNA. These results show that the repair of different kinds of DNA damage can be affected to different extents by some property of this tandemly repeated heterochromatic DNA. To our knowledge, this is the first demonstration in primate cells of differential repair of cellular DNA sequences. (Author abstract) (33 Refs)

38. REASSOCIATION OF HUMAN LYMPHOBLASTOID CELL DNA REPAIR REPLICATED FOLLOWING METHYL METHANESULFONATE TREATMENT.

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Chem Biol Interact; 39(1):77-88 1982

The reassociation rates of repair replicated DNA of two human lymphoblastoid cell lines, the WIL2-A3 'normal' line and the RAJI line of Burkitt's lymphoma, were examined using the DNA/DNA 'C0t' hybridization technique. The cells were treated with methyl methanesulfonate (MMS), an alkylating agent and mutagen, to induce the repair. The incorporated repair replication radioactivity in highly repetitive sequences of WIL2-A3 cell DNA reassociates as expected for a randomly distributed incorporation. The reassociation of repair radioactivity in sequences of fewer numbers of copies, however, is less than expected for a random distribution. It is less than that occurring for semiconservatively synthesized DNA of WIL2-A3 cells co-incubated with the repair labeled DNA as an internal control. The observed difference could be due to an over-representation of repair replication radioactivity in DNA sequences with fewer copies. It is unlikely to be due to residual alkali labile damage resulting from MMS treatment, since a similar difference was not observed when semiconservatively labeled DNA from cells which had been treated with MMS for the same time and at the same concentration as in the repair experiments was substituted for repair replicated DNA in the reassociation reactions. Other possible causes of the apparent difference in the reassociation rates observed are discussed. (Author abstract) (17 Refs)

39. UNSCHEDULED DNA SYNTHESIS INDUCED BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE IN HELA CELLS.

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Gan; 73(1):63-69 1982

N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced repair (unscheduled) DNA synthesis was studied in intact HeLa cells as well as in the cell lysate. The incorporation of

dTTP[methyl-3H] in vitro by the cell lysate was dependent on the dose of MNNG, and was linear for 2 min, reaching the maximum at 4 min. The optimum conditions were the same as for UV-induced in vitro repair synthesis. The presence of hydroxyurea and arabinofuranosyl cytosine during in vivo incubation with MNNG was necessary for enhancement of in vitro DNA synthesis. This MNNG-induced in vitro DNA synthesis was inhibited by aphidicolin, but not by 2',3'-dideoxythymidine-5'-triphosphate. It thus appears that DNA polymerase alpha participates in MNNG-induced unscheduled DNA synthesis in vitro. (Author abstract) (25 Refs)

40. THE ADAPTIVE RESPONSE TO ALKYLATING AGENTS. THE REMOVAL OF O6-METHYLGUANINE FROM DNA IS NOT DEPENDENT ON DNA POLYMERASE-1.

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MRC Cell Mutation Unit, Univ. Sussex, Falmer, Brighton, Sussex BN1 5JH, England
Mutat Res; 104(1-3):67-73 1982

The level of O6-methylguanine (OMG) repair protein was assayed in cell-free extracts of adapted DNA-deficient (PolA) strains of Escherichia coli. Removal of OMG from the DNA of PolA strains in vivo also was examined. The ability to excise OMG from DNA was not impaired in PolA strains due to a defective induction of demethylating activity. Following adaptation, PolA strains produced normal levels of active OMG demethylating protein. Induction and operation of the OMG repair protein seemed normal. (11 Refs)

41. THE USE OF A YEAST STRAIN WITH A TEMPERATURE-SENSITIVE DNA LIGASE TO ESTIMATE DNA REPAIR AFTER EXPOSURE TO MUTAGENS.

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Mutat Res; 104(1-3):79-86 1982

DNA repair in yeast was determined at early times after UV irradiation using the Saccharomyces cerevisiae strain cdc9 that has a temperature-sensitive DNA ligase, by measuring the incidence of single-strand breaks that occur during incomplete repair at the restrictive temperature following UV irradiation. Advantages of the technic are listed. The number of strand breaks induced in cdc9 at 37 C equalled the number of UV-induced pyrimidine dimers at low UV doses. The kinetics of DNA repair following treatment with the chemical mutagen 4-chloromethyl-biphenyl were examined. (10 Refs)

42. EXCISION OF BENZO[A]PYRENE DIOL EPOXIDE I ADDUCTS FROM NUCLEOSOMAL DNA OF CONFLUENT NORMAL HUMAN FIBROBLASTS.

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Chem Biol Interact; 38(3):261-274 1982

The formation and removal of covalent adducts of racemic 7beta,8alpha-dihydroxy-9alpha,10alpha-epoxy-7,8,9,10 tetrahydrobenzo[a]pyrene (BPDE I) was studied in nucleosomal DNA of confluent cultures of normal human fibroblasts (NF). For this purpose NF were prelabeled in their DNA with [14C]-thymidine and treated with [3H]BPDE I. The adducts were composed of 77% (7R)-N2-(7beta,8alpha,9alpha-trihydroxy-7,8,9,10 tetrahydrobenzo[a]pyrene-10-yl)deoxyguanosine, 12% of the corresponding 7S-enantiomer and of minor amounts of adducts to cytosine and adenine. The adduct composition did not change significantly in 24-hr post treatment incubation. Bulk mononucleosomes were prepared from micrococcal nuclease digested nuclei and their DNA analyzed by gel electrophoresis. The adduct concentrations were determined in 145 base pair (bp) nucleosomal core-DNA, 165 bp chromatosomal DNA and in total nuclear DNA. From these data the concentration in nucleosomal linker-DNA was calculated. The initial adduct distribution was non-random and 6.3 times higher in 47 bp linker-DNA relative to 145 bp core-DNA and 9.2 times higher in 27 bp linker-DNA relative to 165 bp chromatosomal DNA. Adduct removal was very rapid during the first 8 hr and more efficient from linker-DNA than from core-DNA. After this early phase the adducts located in 145 bp core-DNA became refractory to further excision and represent a major fraction of the adducts persisting in DNA of NF over a prolonged period. In contrast, further adduct removal was observed from nucleosomal linker-DNA. (Author abstract) (31 Refs)

43. INHIBITORS OF REPAIR DNA SYNTHESIS.

Collins AR, Squires S, Johnson RT
Cancer Res. Campaign Mammalian Cell DNA Repair Group, Dept. Zoology, Univ. Cambridge, Downing St., Cambridge, CB2 3EJ, England
Nucleic Acids Res; 10(4):1203-1213 1982

We have measured repair DNA synthesis in UV-irradiated normal human fibroblasts, grown to a defined state of quiescence in order to avoid the problem of discriminating repair from replication DNA synthesis. We have assessed the effects of various DNA synthesis inhibitors on repair. Inhibition of repair synthesis by hydroxyurea, 1-beta-D-arabinofuranosylcytosine and aphidicolin is associated with the ability to accumulate DNA breaks due to enzymic incision at DNA damage sites; the inhibition by novobiocin is in accord with its known ability to block incision. (Author abstract) (28 Refs)

44. INACTIVATION OF NUCLEOTIDE EXCISION REPAIR IN CHINESE HAMSTER OVARY CELLS BY EXPOSURE TO AN ALKYLATING AGENT.

Park SD, Cleaver JE
(c/o Cleaver) Lab. Radiobiology, Univ. California, San Francisco, CA, 94143
Photochem Photobiol; 35(3):419-421 1982

Unscheduled DNA synthesis and excision of pyrimidine dimers in Chinese hamster ovary cells irradiated with UV light were inhibited by prior exposure to the alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (1-10 uM). Although the pathways for excision of pyrimidine dimers and alkylation damage are known to differ, alkylations from MNNG exposure appear to have a direct effect on the nucleotide excision repair system. These results indicate that the method of exposing cells to two DNA-damaging agents to determine whether they are repaired by common or different pathways can be quite unreliable because of other effects on the repair systems themselves. (Author abstract) (27 Refs)

45. EFFECTS OF APHIDICOLIN ON REPAIR REPLICATION AND INDUCED CHROMOSOMAL ABERRATIONS IN MAMMALIAN CELLS.

van Zeeland AA, Bussmann CJ, Degrassi F, Filon AR, Van Kesteren-van Leeuwen AC, Palitti F, Natarajan AT
Dept. Radiation Genetics and Chemical Mutagenesis, State Univ. Leiden, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands
Mutat Res; 92(1/2):379-392 1982

In growing cultures of normal human skin fibroblasts (HF) and HeLa cells, aphidicolin (APH: 5 ug/ml) enhanced DNA repair replication after UV exposure. In confluent HF cultures, APH inhibited UV-induced repair replication. This inhibitory effect was enhanced in the presence of hydroxyurea (HU: 10 milliM). In confluent HeLa cell cultures, APH inhibited UV-induced repair replication in the presence of HU but enhanced it in the absence of HU. In x-irradiated human peripheral blood lymphocytes (PBL), APH exposure during the G0 or G1 phase increased the occurrence of chromosome breaks and exchanges. The effects of APH on the induction of chromosomal damage in Chinese hamster ovary (CHO) cells depended on the cell cycle stage. In synchronized CHO cells, APH did not significantly affect the frequencies of chromosomal aberrations induced by exposure to x-rays during G2. In G2 cells, APH enhanced the frequencies of x-ray-induced chromatid breaks. In CHO cells exposed to UV, mitomycin C, monofunctional mitomycin C, or 4-nitroquinoline 1-ox ide during G1 and exposed to APH in G2 (just before fixation), the frequencies of chromosomal aberrations were higher than in cells exposed only to UV or the alkylating agents. Despite the difficulty of making direct comparisons between the effects of APH on DNA-repair replication and the frequencies of induced chromosomal aberrations, these results indicate that cell type and cell cycle stage influence the biologic responses to APH. (18 Refs)

46. EFFECT OF POLYAMINES ON ENZYMES INVOLVED IN DNA REPAIR.

Kleppe K, Osland A, Fosse V, Male R, Lossius I, Helland D, Lillehaug JR, Raae AJ, Kleppe RK, Nes IF
Dept. Biochemistry, Univ. Bergen, Bergen, Norway
Med Biol; 59(5/6):374-380 1981

The effects of polyamines on UV incision nuclease, DNA polymerase I, DNA ligase and polynucleotide kinase, apurinic- or apyrimidinic (AP)- endonucleases, and enzymes involved in folding and unfolding of chromosomes are reviewed. Polyamine-induced cleavage of AP-sites is also discussed. Spermine and spermidine have been found to be more efficient in causing hydrolysis of DNA than cadaverine or putrescine. (29 Refs)

47. POTENTIATION OF CELL KILLING BY INHIBITORS OF POLY(ADP-RIBOSE) POLYMERASE IN FOUR RODENT CELL LINES EXPOSED TO N-METHYL-N-NITROSOUREA OR UV LIGHT.

Durrant LG, Boyle JM
Paterson Lab., Christie Hosp. and Holt Radium Inst., Manchester M20 9BX, England
Chem Biol Interact; 38(3):325-338 1982

The sensitivities (Do-values) of the cytotoxic effect of MNU on four rodent cell lines were: mouse L1210, 0.07 mM; rat Yoshida sarcoma, 0.52 mM; Chinese hamster V79A, 0.70 mM and the UV sensitive, x-ray sensitive V79/79, 0.35 mM. The abilities of maximum non-toxic doses of the poly(ADP-ribose) polymerase inhibitors, 5-methyl nicotinamide (5MeN), 3-methoxybenzamide (3MBA) and caffeine to potentiate this cytotoxicity and that of UV light in V79A and V79/79 was measured. The degree of potentiation (ratio Do without inhibitor/Do with inhibitor) was both agent and cell line dependent. In general the lymphoid cell lines L1210 and YS showed greater potentiation, up to four-fold, than did the fibroblast lines V79A and V79/79. The use of inhibitors in pairs suggested that 5MeN and 3MBA affect one process whereas caffeine affects additional processes. The data provide further support for a role for poly(ADP-ribose) in DNA repair, but indicate that metabolic factors may modify the effectiveness of individual inhibitors of poly(ADP-ribose)polymerase in different cell lines. (Author abstract) (40 Refs)

48. REGULATION OF DNA LIGASE ACTIVITY BY POLY(ADP-RIBOSE).

Creissen D, Shall S
Cell and Molecular Biology Lab., Univ. Sussex, Brighton, Sussex BN1 9QG, England
Nature; 296(5854):271-272 1982

Nuclear ADP-ribosyl transferase (ADPRT) catalyzes transfer of ADP-ribose from NA D+ to chromatin proteins to form mono, oligo- and poly(ADP-ribose) modified chromatin proteins. Various other studies are reviewed which suggest that (ADP-ribose)n biosynthesis is necessary for effective repair of some types of DNA damage. The question remains as to which step in DNA repair requires ADPRT activity. Experiments are described which support the hypothesis that ADP-ribosylation activates DNA ligase activity. It is suggested that ADPRT has a general function in the control of the breaking and rejoining of DNA. (46 Refs)

49. EFFECT OF HEAT SHOCK ON POLY(ADP-RIBOSE) SYNTHETASE AND DNA REPAIR IN DROSOPHILA CELLS.

Nolan NL, Kidwell WR
Lab. Pathophysiology, NCI, NIH, Bethesda, MD, 20205
Radiat Res; 90(1):187-203 1982

The capacity of Drosophila cells to repair gamma-ray induced DNA lesions using the quantity of poly(ADP-ribose) synthetase (PARS) left after heat shock (37 C) was investigated. PARS was not necessary for rejoining gamma-ray induced DNA strand breaks. Inhibitors of PARS, 3-aminobenzamide and 5-methyl nicotinamide, lowered the PARS level in whole cells and prevented an increase in polymer levels following irradiation, without blocking the rejoining of gamma-ray-induced DNA strand breaks. (58 Refs)

50. REPAIR OF DOUBLE-STRAND DNA BREAKS IN DROSOPHILA.

Dezzani W, Harris PV, Boyd JB
Dept. Genetics, Univ. California, Davis, CA, 95616
Mutat Res; 92(1/2):151-160 1982

Repair kinetics of DNA double-strand breaks (DSB) in primary and established cell cultures of Drosophila melanogaster were examined. In primary cultures, 50% of the DSB induced by x-rays (10,000 R) were repaired within 45 min, and 80% were repaired within 2-3 yr. Similar DSB repair kinetics were noted in established Drosophila cell cultures. The half-time for DSB repair was shorter in the established cultures (20 min) than in the primary cultures (40 min). Comparison of the results with previously published data suggests that DSB repair in primary Drosophila cell cultures may resemble that of L1210 cells, and in established Drosophila cell cultures repair may be more rapid than in L1210 cells. Nine mutant Droso-

phila cell cultures with deficiencies in other types of DNA repair (including meiotic mutants mei-9a-2, mei-41D5, and mei-218) showed no evidence of deficient DSB repair after x-ray exposure. (30 Refs)

51. REPAIR OF ULTRAVIOLET-DAMAGED TRANSFORMING DNA IN A MISMATCH REPAIR-DEFICIENT STRAIN OF HAEMOPHILUS INFLUENZAE.

Bagci H, Stuy JH
(c/o Stuy) Dept. Biological Science, Florida State Univ., Tallahassee, FL, 32306
Photochem Photobiol; 35(3):331-335 1982

Ultraviolet inactivation of Haemophilus influenzae transforming DNA followed inverse square root kinetics in both mismatch repair-proficient (hex+) and deficient (hex-1) recipients. No DNA concentration effect was seen with UV-excision repair-deficient (uvr-) strains. Low-efficiency genetic markers remained more sensitive than high-efficiency ones when they were assayed on excision repair-deficient hex+ uvr- strains. They were equally resistant when hex- uvr- recipients were used. We explain this by assuming that recombinational repair of UV lesions in the donor strand and mismatch repair of the recipient strand may overlap and cause double strand interruptions. This will eliminate low-efficiency transformants. (Author abstract) (35 Refs)

52. CHARACTERIZATION OF POSTREPLICATION REPAIR IN SACCHAROMYCES CEREVISIAE AND EFFECTS OF RAD6, RAD18, REV3 AND RAD52 MUTATIONS.

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Dept. Radiation Biology and Biophysics, Univ. Rochester, Sch. Medicine and Dentistry, Rochester, NY, 14642
MGG; 184(3):471-478 1981

Postreplication repair of nuclear DNA was examined in an excision defective haploid strain of yeast lacking mitochondrial DNA (rad1 p(0)). The size of the DNA synthesized in cells exposed to various fluences of ultraviolet light (UV) corresponds approximately to the av interdimer distance in the parental DNA. Upon further incubation of cells following exposure to 2.5 J/m2, the DNA increases in size; by 4 hr, it corresponds to DNA from uniformly labeled cells. The alkaline sucrose sedimentation pattern of DNA pulse labeled at various times after UV irradiation, for up to 4 hr, does not change substantially, indicating that dimers continue to block DNA replication. A significant amount of postreplication repair requires de novo protein synthesis, as determined by its inhibition by cycloheximide. The rad6 mutant does not carry out postreplication repair, the rad18 and rad52 mutants show great inhibition while the rev 3 mutation does not affect postreplication repair. Both recombinational and nonrecombinational repair mechanisms may function in postreplication repair and most of postreplication repair is error free. (Author abstract) (54 Refs)

53. A NEW PATHWAY FOR REPAIR OF CROSS-LINKABLE 8-METHOXYPSORALEN MONO-ADDUCTS IN UVR- STRAINS OF ESCHERICHIA COLI.

Bridges BA, Stannard M
MRC Cell Mutation Unit, Univ. Sussex, Falmer, Brighton, Sussex, BN1 9QG, England
Mutat Res; 92(1/2):9-14 1982

In three Uvr-families of Escherichia coli, a metabolic process was discovered involving certain mono-adducts of 8-methoxypsoralen (8-MOP) in DNA, leading to a loss of their ability to form lethal cross-links after UV exposure, removal of unbound 8-MOP, and another exposure to near UV after a variable recovery period. This process was dependent on the polA+ gene (DNA polymerase), but not on protein synthesis or DNA replication. It seemed to be a repair pathway, independent of uvrAB endonuclease, initiated either by a different endonuclease or an N-glycosylase. This repair process was not affected by a rep gene mutation and thereby differed from the uvrA+-independent cross-link repair pathway, but the results did not exclude the possibility that a common enzyme may be involved. (12 Refs)

54. VALIDATION OF A DNA-REPAIR SYNTHESIS ASSAY ON POOLS OF FRESH AND FROZEN-THAWED LYMPHOCYTES.

Capelli E, Stefanini M, Giulotto E, Nuzzo F
Istituto di Genetica Biochimica ed Evoluzionistica del C.N.R., Via S. Epifanio 14, 27100 Pavia, Italy
Mutat Res; 104(1-3):187-191 1982

The possibility of measuring [3H]thymidine incorporation (HTI) stimulated by various treatments was studied in human lymphocyte pools, both fresh and thawed, after storage in liquid nitrogen. For each experiment cells obtained from 4-6 blood samples were mixed in equal proportions prior to exposure. Reagent grade purity chemicals and ranges tested were ethyl methanesulfonate (EMS) 5-20 milliM (mM), methyl methanesulfonate (MMS) 0.5-4 mM, epichlorhydrin (ECCH) 10(-4)-10(2) mM, mitomycin C (MC) 10(-6)-10(2) mM, hycanthone (HY) 10(-2)-2 mM, and cycloheximide (CHX) 3.5 x 10(-3)-3.5 x 10(-2) mM. Repair synthesis was expressed as the difference between HTI values in the presence of hydroxyurea (HU) in treated and untreated cells. CHX reduced HTI without any stimulation of repair. MMS, EMS, and ECCH induced repair synthesis at various concentrations, irrespective of the degree of inhibition of duplicative synthesis. HY inhibited DNA synthesis and induced a slight HTI increase only at 0.1 mM. MC reduced DNA synthesis at the highest concentration assayed and did not significantly increase the HTI rate in the presence of HU. Mean values of repair synthesis obtained with agents in different experiments are presented. The mean cell viability after thawing was 97.2% and decreased with incubation time (64.4% after 24 hr at 37 C). Repair synthesis activity was always present. HTI values in irradiated cells, both in the presence and absence of HU were always higher than in control cells. Results indicate that pools human lymphocytes can be used as an alternative to heteroploid cell lines and diploid fibroblasts in DNA-repair assays. (13 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

55. INHIBITION OF PM-2 DNA DEGRADATION BY A HUMAN SERUM PROTEIN.

Galvan L, Evans JE, Huang CH, Prestayko A, Wu B, Crooke ST
(c/o Crooke) Dept. Pharmacology, Baylor Coll. Medicine, Houston, TX, 77030
Cancer Res; 42(4):1555-1561 1982

A unique DNA-binding protein was detected that inhibited DNA degradation induced by bleomycin and was decreased in sera of cancer patients. The protein from normal human serum was purified to homogeneity by ammonium sulfate precipita-

tion and DEAE-cellulose and DNA-cellulose column chromatography. Two-dimensional isoelectric focusing gel electrophoresis revealed a single protein spot with a mol wt of 64,000 and a pI at pH 5.9. The NH2 terminus was lysine, and the ratio of acidic to basic residues was 1.2. DNA binding was demonstrated by column chromatography, agarose gel electrophoresis, fluorescence quenching, and circular dichroism. The inhibitory activity was abolished by treatment with Pronase but not by RNase or DNase I. FeCl2 caused a partial loss of inhibitory activity. The inhibition of DNA degradation was more effective for breakage induced by bleomycin than neocarzinostatin, macromomycin, or DNase I. Evidence from DNA-binding studies suggests the inhibition is due to binding of the protein to sites on DNA preferred by bleomycin. Thus, the protein could be useful for studies on the mechanism of action of bleomycin and other antitumor agents, the cytotoxic effects of which are due primarily to damage of cellular DNA. The protein was decreased significantly in sera of cancer patients, and its potential use as a diagnostic tool for neoplasias is being investigated further. (Author abstract) (26 Refs)

56. GENOME INSTABILITY IN A REGION OF HUMAN DNA ENRICHED IN ALU REPEAT SEQUENCES.

Calabretta B, Robberson DL, Barrera-Saldana HA, Lambrou TP, Saunders GF
Dept. Biochemistry, Univ. Texas System Cancer Center, Houston, TX, 77030
Nature; 296(5854):219-225 1982

DNA rearrangements involving restriction fragment length polymorphism and variation in copy number were detected in the human genome by blot hybridization with a cloned segment of human DNA initially present in a cluster of Alu repeat sequences. These rearrangements involve both extrachromosomal circular duplex DNAs and integrated sequences, indicating the presence of transposable elements in human cells. (Author abstract) (29 Refs)

57. MODIFICATIONS IN ALPHA 2U GLOBULIN GENE STRUCTURE, TRANSCRIPTION, AND MRNA TRANSLATION IN HEPATOMAS.

Nakhasi HL, Lynch KR, Dolan KP, Unterman R, Antakly T, Feigelson P
(c/o Feigelson) Dept. Biochemistry, Inst. Cancer Res., Columbia Univ., New York, NY, 10032
J Biol Chem; 257(6):2726-2729 1982

Alpha 2u globulin synthesis ceases when a liver cell becomes malignant. We have compared the structure and transcription of the alpha 2u globulin genes in Morris hepatomas 5123D and 7793 with that of normal hepatic genes using alpha 2u globulin cDNA as a hybridization probe. No alpha 2u globulin mRNA was detected in hepatoma 7793 by cell-free translation, Northern blot, or Rot analysis. In hepatoma 5123D, however, a small number of alpha 2u globulin RNA sequences were detected by Northern blot and Rot analysis, but this RNA was not detectably translationally active either in vivo or in an in vitro cell-free translational system. No structural differences between normal liver and the hepatoma alpha 2u globulin genes were observed by Southern blot analysis of genomic DNA digested with restriction endonucleases Ps t I, Bam HI, or Ava I. However, a general demethylation of cytosine residues in the alpha 2u globulin genes of hepatomas has been found using the restriction enzyme Hha I and the isoschizomeric pair of restriction enzymes Msp I and Hpa II. Therefore, loss of the alpha 2u globulin phenotype in these hepatomas is accompanied by extensive demethylation of DNA sequences within and/or immediately flanking the alpha 2u globulin genes. (Author abstract) (29 Refs)

58. RECOMBINED FLANKS OF THE VARIABLE AND JOINING SEGMENTS OF IMMUNOGLOBULIN GENES.

Hochtl J, Muller CR, Zachau HG
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Proc Natl Acad Sci USA; 79(5):1383-1387 1982

The mechanism of generating immunoglobulin light chain genes by rearrangement of variable (V), joining (J), and constant (C) gene segments is still unknown. It has been discussed mostly in terms of excision and deletion of the DNA between the recombined V and J gene segments. However, the finding in DNA digests from the mouse myeloma T of a fragment (called f-T) that contains the 3' flank of a V kappa and a 5' flank of a J1 gene segment argued against a simple deletion mechanism. The origin of fragment f-T has now been investigated by cloning and determining the sequence of the germline V gene segment that apparently participated in its formation. Moreover, analogous fragments containing flanking sequences were isolated from the myelomas MOPC 173 and 41 (f-173 and f-41) and studied by sequence analysis. The f fragments appear to be recombination products of V-J rearrangements reciprocal to rearranged kappa genes but, at least in the cases of f-T and f-173, not of the rearranged V genes present in the same tumor cell. This fact is best explained by a sister chromatid exchange mechanism of V-J recombination because, by this model, the rearranged V genes and the reciprocal flank recombination products would segregate into different cells during the following mitosis. The possibility is suggested that there exists in lymphocyte differentiation more than one mechanism of V-J recombination. (Author abstract) (27 Refs)

59. ISOLATION AND CHARACTERIZATION OF A HUMAN LOCUS HOMOLOGOUS TO THE TRANSFORMING GENE (V-FES) OF FELINE SARCOMA VIRUS.

Trus MD, Sodroski JG, Haseltine WA
Lab. Biochemical Pharmacology, Sidney Farber Cancer Inst., Boston, MA, 02115
J Biol Chem; 257(6):2730-2733 1982

A single locus (designated c-fes) in the human genome which exhibits homology to the transformation-specific onc gene (v-fes) of Snyder-Theilen feline sarcoma virus was identified by the Southern blot technique. Recombinant clones containing 16-to 18-kilobase inserts of human DNA including the c-fes locus were constructed. Restriction endonuclease mapping of these clones verified their ide ntity with native human c-fes and demonstrated the presence of at least two sequences in human c-fes interrupting v-fes-homologous regions. The v-fes-homologous locus in the human genome spans about 4 kilobases. The 5'-3' orientation of the c-fes clones with respect to feline sarcoma virus proviral DNA was determined. The region of the human genome that is homologous to v-fes is proximal to the highly reiterated human Alu sequence but not to the highly reiterated human alphoid sequence. (Author abstract) (39 Refs)

60. COMPARISON OF THE SEQUENCES AT SPECIFIC SITES ON DNA CLEAVED BY THE ANTITUMOR ANTIBIOTICS TALISOMYCIN AND BLEOMYCIN.

Mirabelli CK, Beattie WG, Huang CH, Prestayko AW, Crooke ST
Bristol-Baylor Lab., Dept. Pharmacology, Baylor Coll. Medicine, Texas Medical Center, Houston, TX, 77131
Cancer Res; 42(4):1399-1404 1982

We have investigated the site-specific cleavage of DNA by the antitumor antibiotics talisomycin and bleomycin by using 5'- or 3'-terminal 32P-labeled restriction fragments of pBR.322 DNA. Both drugs cleaved DNA preferentially at G-C and G-T sequences. However, the relative amounts of cleavage at particular cleavage sites differed between talisomysin and bleomycin at concentrations of the drugs which produced similar extents of total cleavage. In addition, talisomycin produced specific cleavages at G-A sequences which were relatively resistant to cleavage by bleomycin. Within a preferred sequence group (ie, G-C sequences), some sites were cleaved to a greater extent relative to others by both talisomycin and bleomycin, suggesting that a greater degree of specificity than that provided by only two nucleotides is involved in the site-specific recognition and cleavage of DNA by these drugs. (Author abstract) (29 Refs)

61. CHROMOSOME STRUCTURE AND DNA SEQUENCE ALTERATIONS ASSOCIATED WITH MUTATION OF TRANSFORMED GENES.

Robins DM, Axel R, Henderson AS
Inst. Cancer Res., Coll. Physicians and Surgeons, Columbia Univ., 701 West 168th St., New York, NY, 10032
J Mol Appl Genet; 1(3):191-203 1981

Four well-characterized transformed BRL rat liver cell lines, containing the viral tk gene (tk+) and 5-10 human growth hormone (HGH) sequences, and revertant sublines lacking the tk gene (tk-), were analyzed for the presence of tk and HGH sequences by blot hybridization to restriction endonuclease-cleaved DNA. Some of the tk- revertants were also characterized by karyotypic analysis and hybridization in situ. Over 50% of 40 tk- revertants had deleted all of the transforming DNA from the recipient chromosome. In 15 other revertants, a significant amount of deletion was observed but some of the transforming DNA was retained. The deletion of transforming DNA was never associated with the loss of an entire chromosome. In this restricted set of mutants, it seemed to be crucial to maintain a diploid chromosomal complement. T his condition might be required for the survival of somatic cells in which essential genes are often present in a functionally hemizygous state. Alternatively, subtle gene dosage effects might require a balance associated with diploidy. (18 Refs)

62. AMPLIFIED DNA SEQUENCES IN Y1 MOUSE ADRENAL TUMOR CELLS: ASSOCIATION WITH DOUBLE MINUTES AND LOCALIZATION TO A HOMOGENEOUSLY STAINING CHROMOSOMAL REGION.

George DL, Powers VE
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Proc Natl Acad Sci USA; 79(5):1597-1601 1982

DNA associated with double minutes (dm) of the Y1-DM mouse adrenocortical tumor cell line has been cloned in Charon 4A and a preliminary characterization has been made of a recombinant clone, lambdaY1dm-1, isolated from this dm DNA library. Cloned sequences in lambdaY1dm-1 are amplified in the genome of the Y1-DM cells. They are also amplified in the genome of a related Y1 subline (Y1-HSR), which has a homogeneously staining chromosomal region (HSR). Here we report that the amplified sequences complementary to lambdaY1dm-1 are localized to the HSR, as determined by in situ hybridization. In addition, we found that a population of Y1-DM cells originally containing only dm later consisted of two cell types. Some cells retained dm; others had lost dm but gained a HSR-bearing chromosome morphologically distinct from that in the Y1-HSR cell line. Subclones isolated from this mixed culture have either dm or a HSR, but not both. Southern blotting studies revealed that genomic DNA samples from subclones with a HSR, like subclones with dm, still possess amplified copies of DNA homologous to our recombinant probe. These experiments provide direct evidence that the dm and HSRs in these Y1 cells are structurally related and further support the hypothesis that these chromosomal anomalies result from a process of gene amplification. (Author abstract) (25 Refs)

63. HELA CELLS LACK RECA GENE.

Mishra NC
Dept. Biology, Univ. South Carolina, Columbia, SC, 29208
FEBS Lett; 137(2):175-177 1982

Hybrid plasmids (pBR322) containing 32P-labeled recA gene of Escherichia coli were quantitatively hybridized with HeLa cell DNA, using a procedure combining agar gel electrophoresis and Southern transfer. HeLa cell DNA does not contain a gene homologous to the recA gene of E coli. Either the recA gene is absent in higher eukaryotes or the DNA sequences between the two organisms studied are too divergent. (14 Refs)

64. ULTRAVIOLET SENSITIVITY OF THE ADDITION, DELETION AND REPLACEMENT OF LONG NONHOMOLOGOUS DNA SEGMENTS BY GENETIC TRANSFORMATION OF HAEMOPHILUS INFLUENZAE.

Walter RB, Stuy JH
(c/o Stuy) Dept. Biological Science, Florida State Univ., Tallahassee, FL, 32306
Photochem Photobiol; 35(3):337-341 1982

We have recently described the construction and some properties of Hemophilus influenzae Rd strains with long and different R plasmid-derived DNA segments (nonhomologous inserts) at the same site in the HP1 prophage. These inserts can be added to a recipient's genome by genetic transformation, they can be deleted from the recipient genome, or they can be replaced by another insert. We report here that the UV inactivation of all three phenomena followed single hit kinetics. Deletion was roughly 10x more resistant; its UV-sensitivity equaled that of a high-efficiency point mutation. There was an inverse correlation between UV-sensitivity and additive transformation efficiency of the various inserts; sensitivity may thus be a measure of insert size. This correlation was not seen for deletion. All three phenomena were more sensitive when they were measured on excision repair-deficient uvr- recipients. The dose-reduction factor for addition was about 1.5 while it was about 2.6 for deletion. (Author abstract) (10 Refs)

65. PRELIMINARY CHARACTERIZATION OF THE ADDUCTS FORMED BETWEEN THE ANTITUMOR COMPOUND CIS-PT(NH3)2CL2 AND DNA.

Johnson NP
Laboratoire de Pharmacologie et de Toxicologie Fondamentales, Centre National de la Recherche Scientifique, 205 route de Narbonne, 31400 Toulouse, France
Biochem Biophys Res Commun; 104(4):1394-1400 1982

Electrophoresis of the products formed by the reaction of cis-Pt(NH3)2Cl2 (cP) with DNA gave a profile of Pt concentration which contained five peaks whose relative intensity varied with the amount of cP fixed on the DNA. Similar study with two Pt compounds which lacked antitumor activity showed a different manner of binding to DNA. DNA isolated from Escherichia coli which had been treated with cP or [Pt(dien)Cl]Cl did not yield the same profiles on electrophoresis as the corresponding Pt-DNA compounds formed in vitro. (10 Refs)

66. BENZPYRENE GROUPS BIND PREFERENTIALLY TO THE DNA OF ACTIVE CHROMATIN IN HUMAN LUNG CELLS.

Arrand JE, Murray AM
Dept. Biochemistry, St. Mary's Hosp. Medical Sch., London W2 1PG, England
Nucleic Acids Res; 10(5):1547-1555 1982

The cells of the bronchial epithelium of man are targets for benzo(a)pyrene carcinogenesis. When cultures of these cells, and of non-target fibroblasts, are exposed to [3H]benzo(a)pyrene, we find that the epithelial cells metabolize and bind to DNA far greater amounts of benzpyrene than do fibroblasts. By analysis of nuclei of benzpyrene-treated cells for sensitivity to limited digestion with pancreatic DNase I, we have shown that benzpyrene groups bind initially to the DNA of expressed (DNase I sensitive) regions of chromatin in both cell types. Covalent binding of benzpyrene groups to nonexpressed (DNase I resistant) regions follows rapidly in the target epithelial cells. These maintain high levels of carcinogen adducts in their DNA. In fibroblasts, benzpyrene group binding to non-expressed DNA occurs more slowly and active removal of adducts from the DNA is evident. (Author abstract) (21 Refs)

67. NONDETECTION OF O(6)-METHYLGUANINE IN RAT DNA FOLLOWING IN VIVO TREATMENT WITH LARGE DOSES OF CIMETIDINE ALONE OR IN COMBINATION WITH SODIUM NITRITE.

Kyrtopoulos SA, Hadjiloucas E, Vrotsou B
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Cancer Res; 42(5):1962-1966 1982

Cimetidine was administered by stomach tube to rats at 70 or 700 mg/kg, doses corresponding to 5 or 50 times, respectively, the typical daily dose of individuals on cimetidine treatment. In some cases, cimetidine (70 mg/kg) was administered in combination with a two-fold molar excess of sodium nitrite. This treatment was carried out up to six times over a 3-day period, the pH of the rat stomach being maintained at 2.3 to 3.0 for about 1 hr after each treatment. The DNA of the stomach, liver, and intestines (large and small pooled together) were isolated 6 hr after cessation of treatment and analyzed for the presence of O6-methylguanine using a sensitive and specific radioimmunoassay. No evidence could be obtained for the presence of this methylated base in any of the DNA samples examined, the limit of detection being 3 umol O6-methylguanine per mol guanine. We suggest that the observed lack of DNA methylation may be primarily due to the slow rate of nitrosation of cimetidine in combination with its rapid absorption into the blood stream. (Author abstract) (38 Refs)

68. MURINE ERYTHROLEUKEMIA CELL DIFFERENTIATION: DNASE I HYPERSENSITIVITY AND DNA METHYLATION NEAR THE GLOBIN GENES.

Sheffery M, Rifkind RA, Marks PA
Lab. Developmental Cell Biology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY, 10021
Proc Natl Acad Sci USA; 79(4,part1):1180-1184 1982

The sensitivity to digestion by DNase I of chromatin containing the alpha- and betamajor-globin genes and the pattern of DNA methylation near these genes was examined during hexamethylenebisacetamide (HMBA)-mediated erythroid differentiation of murine erythroleukemia cells (MELC). In uninduced and induced cells, the chromatin regions containing the alpha- and betamajor-globin genes are more sensitive to digestion by DNase I than is the region containing an immunoglobulin gene (Igalpha) not expressed during erythroid differentiation. However, at low concentrations of DNase I, a 6-to 10-fold increase in site-specific cleavages was generated in chromatin regions near both the alpha- and betamajor-globin genes in cells induced to differentiate by HMBA. The DNase I hypersensitive site near the betamajor-globin gene maps to a small region near the 5' terminus of the gene. No detectable change in the pattern of DNA methylation around either the alpha- or beta-globin genes was observed during HMBA-mediated erythroid differentiation. Of the potentially methylated sites assayed and mapped near the betamajor-globin gene, one site is fully methylated, one is partially methylated, and one is unmethylated both in uninduced and induced cells. Many (but not all) sites assayed near the alpha-globin genes are unmethylated in both uninduced and induced cells. These results show that specific alterations of chromatin structure occur during MELC differentiation and suggest that these changes may not involve alterations in the pattern of DNA methylation. (Author abstract) (35 Refs)

69. DNA METHYLATION LEVELS IN NORMAL AND CHEMICALLY-TRANSFORMED MOUSE 3T3 CELLS.

Diala ES, Hoffman RM
Dept. Pediatrics, M-009, Univ. California at San Diego Sch. Medicine, La Jolla, CA, 92093
Biochem Biophys Res Commun; 104(4):1489-1494 1982

Normal mouse embryo 3T3 cells grown in culture and cells transformed by benzo(a)pyrene and methylcholanthrene were analyzed by high performance liquid chromatography (HPLC) to determine the 5-methylcytosine to cytosine base ratios in their total genomic DNA. The DNA methylation levels were approx equal in the three cell lines. Findings obtained with HPLC and those obtained by other techniques are discussed. (16 Refs)

70. ELEVATED LEVEL OF ENZYMATIC DNA METHYLATION IN CELLS TREATED WITH 1-BETA-D-ARABINOFURANOSYLCYTOSINE.

Boehm TL, Drahovsky D

(c/o Drahovsky) Zentrum der Biologischen Chemie, Universitat Frankfurt, Theodor Stern-Kai 7, D-6000 Frankfurt am Main 70, W. Germany

Cancer Res; 42(4):1537-1540 1982

When mouse P815 mastocytoma cells are cultured in the presence of 1-beta-D-arabinofuranosylcytosine (ara-C), a dose-dependent inhibition of cell proliferation and DNA synthesis can be observed. The DNA synthesized in the presence of ara-C is more highly enzymatically methylated than that of controls as measured by conversion of incorporated [14C]deoxycytidine-derived radioactivity into 5-methylcytosines. Analysis of DNAs by means of the bacterial restriction enzyme Hpall and its isoschizomer Mspl revealed an aberrant DNA methylation pattern after ara-C treatment. The hypermethylation of DNA persisted also in the cell cycle following the treatment in the absence of ara-C. No such changes in DNA methylation patterns following ara-C treatment were observed in P815/ara-C, a subclone of P815 cells resistant to ara-C. (Author abstract) (22 Refs)

71. CONFORMATION OF 2-[(DEOXYGUANOSIN-8-YLACETYL)AMINO]FLUORENE DIFFERS IN PROTEIN-FREE DEOXYRIBONUCLEIC ACID AND CHROMATIN.

Ross J, Metzger G, Werbin H

(c/o Werbin) Programs in Biology, Univ. Texas at Dallas, Richardson, TX, 75080

Biochemistry; 21(6):1369-1374 1982

There is substantial evidence that modification of protein-free DNA with 2-[(ace toxyacetyl)amino]fluorene results in the formation of 2-[(deoxyguanosin-8-ylacetyl)amino]fluorene adducts in which the fluorene ring inserts between the bases and the guanosine is shifted to the outside of the helix creating localized regions of denaturation. It was therefore of interest to determine the conformation of similarly induced adducts in chromatin and nucleosome core particles by circular dichroism, thermal denaturation, and single-strand-specific endonuclease susceptibility. Fluorenyl residues in modified chromatin and core particles gave rise to a less intense circular dichroism signal at 300 nm than those in purified DNA: approx-190 vs approx-280 deg cm2/nmol of fluorene residues, respectively. Levels of modification from 1.37 to 2.87 fluorenyl residues/100 nucleotides that produced a decrease of the melting temperature from 0.8 to 2.0 C in protein-free DNA had no effect upon the melting temperature of core particles. Adducts were not removed from modified core particles and chromatin by Neurospora crassa endonuclease as they were from modified DNA, even though this enzyme at the same concentration (12 units/A260 unit) recognized single-stranded regions introduced into the core particles by mild DNase I digestion. These data demonstrate minimal interaction of the fluorenyl residues with the DNA helix and provide no evidence for their induction of locally denatured regions. Hence, the existence of different conformations for modified bases in chromatin and protein-free DNA is proposed. The implications of this nondenaturing conformation with respect to the in vivo repair of these lesions are discussed, as well as the possibility that modified bases adopt a Z-DNA-like structure in modified chromatin as they do in modified poly(dG-dC)-poly(dG-dC). (Author abstract) (38 Refs)

OTHER RELATED STUDIES

72. NEW METHODS FOR ANALYSING DNA MAKE GENETICS SIMPLER.

Southern EM

MRC Mammalian Genome Unit, Dept. Zoology, Univ. Edinburgh, West Mains Road, Edinburgh EH9 3JT, Scotland

Biochem Soc Trans; 10(1):1-4 1982

Many difficulties in fine-structure mapping in higher organisms are overcome by direct analysis of genes at the molecular level. The possibilities presented by new methods are illustrated by two examples: analysis of linkage of the histone genes in the sea urchin and analysis of globin genes in man. The first example shows how the analysis is done and used where the classical approach would have been impossible. The second compares results of applying the two different approaches to the same set of genes. Also discussed is the potential of the methods for analyzing genetic disorders in man. (14 Refs)

73. MODULATION OF ASCITES TUMOUR GROWTH BY NUCLEIC ACIDS AND NUCLEASES.

Burdon RH, Sillar GM, Allen E

Dept. Biochemistry, Univ. Glasgow, Glasgow G12 8QQ, Scotland

Br J Cancer; 45(2):295-299 1982

Effects of DNA and RNA and DNase and RNase on ascites tumor growth in vitro and in vivo were investigated. The considerable cell loss occurring during development of the Krebs 2 tumor may cause the high levels of DNA and RNA fragments in ascitic fluid. Addition of 300 ug/ml DNA (calf thymus) beneficially affected growth of tumor cells from 2-day-old tumors; prior treatment of the added DNA with DNase I abolished stimulation and heat denaturation of the DNA reduced stimulation. The ability of cells from 2-day-old tumors to grow in culture following pretreatment with high levels of electrophoretically purified DNase I sufficient to digest DNA of most of the nucleoprotein complexes in the extracellular ascites fluid was studied. Such treatment depressed the growth of tumor cells. Pretreatment with high levels of RNase A also depressed growth of tumor cells. Addition of 1 mg/ml DNase I, 2 mg/ml RNase, or 300 ug/ml DNA had no significant effect on growth of 3-day cultured L-929 mouse fibroblasts. Extent of tumor growth in vivo was measured by relative wt gain in mice inoculated with Krebs 2 tumor. Tumor growth was enhanced by the addition of 100 ug portions of DNA ip; RNA inoculation had a varied and less pronounced stimulatory effect. Pretreatment of tumor inoculum with DNase or RNase resulted in subsequent body wt gain, but only half the normal. Tumor development was retarded by successive administration of RNase or DNase after a delay of 24 hr after inoculation of tumor cells; a delay of 7 days reduced tumor growth to half. RNase was always slightly more effective than DNase in the in vivo tests, perhaps because DNase is labile in ascites fluid at 37 C. (14 Refs)

74. IN VIVO AND IN VITRO EFFECTS OF CIGARETTE SMOKE ON CHROMOSOMAL DAMAGE AND SISTER-CHROMATID EXCHANGE IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES.

Vijayalaxmi, Evans HJ
Medical Res. Council, Clinical and Population Cytogenetics Unit, Western General Hosp., Crewe Road, Edinburgh EH4 2XU, Scotland
Mutat Res; 92(1/2):321-332 1982

The frequencies of chromosomal aberrations in phytohemagglutinin-stimulated peripheral blood lymphocytes (PBL) were studied in 49 women and 47 men (20-75 yr old); 55 were smokers (mostly in the light-to-moderate range, 1-10 cigarettes/day) and 41 were nonsmokers. The mean frequencies of all chromosomal aberrations combined were significantly higher in the smokers than in the nonsmokers (3.20 vs 2.22%). These differences were greatest for the frequencies of interchanges (0.22 vs 0.02%), gaps (0.56% vs 0.24%), fragments (0.67 vs 0.34%), dicentrics (0.42 vs 0.14%), and unambiguous exchange-type aberrations (0.64 vs 0.17%). A comparison of PBL cultures from a small group of subjects (3 nonsmokers, 3 heavy smokers) showed a 50% higher baseline frequency of sister chromatid exchanges (SCEs) in the smokers (means, 11.6 vs 7. 6 SCEs/cell). Exposure to cigarette smoke condensate (CSC: 0.1-1.5 mg/10 ml) caused dose-dependent increases in SCE frequencies in the PBL of both smokers and nonsmokers. The dose-response curves were parallel, suggesting that the apparently greater effects of CSC in causing SCEs in the PBL of smokers simply reflected the higher basal SCE frequencies in this group, although the smokers' PBL showed somewhat more unscheduled DNA synthesis in response to CSC than the nonsmokers' PBL. Mitomycin C (10(-8)-10(-7) M) and ethyl methanesulfonate (10(-4)-10(-3) M) caused dose-dependent increases in the SCE frequencies in PBL from both smokers and nonsmokers. The parallel dose-response curves noted with both agents in both types of PBL suggested that the results reflected the basal differences in SCE frequencies between the groups, as opposed to a greater intrinsic sensitivity to mutagens in the PBL of smokers. (19 Refs)

75. 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA) INDUCES SISTER-CHROMATID EXCHANGES AND DELAYS IN CELL PROGRESSION IN CHINESE HAMSTER OVARY AND HUMAN CELL LINES.

Schwartz JL, Banda MJ, Wolff S
Lab. Radiobiology, Univ. California, San Francisco, CA, 94143
Mutat Res; 92(1/2):393-409 1982

In Chinese hamster ovary (CHO) cells and SV40-transformed human fibroblast cell lines (GM637 and XP12RO), 12-O-tetradecanoylphorbol-13-acetate (TPA: 1.0 nanog-1.0 ug/ml) caused a 20-45% increase in the frequency of sister chromatid exchanges (SCEs). This increase in SCEs was not caused by an impurity in the TPA preparation, or increased 5-bromo-2'-deoxyuridine uptake into DNA; it was independent of the incubation time between TPA exposure and fixation of the cells. Simultaneous exposure to TPA (1.0 ug/ml) and benzo(a)pyrene (BP: 1.7 x 10(-5)-3.3 x 10(-6) M) led to a synergistic SCE induction in CHO cells. No synergism was observed after simultaneous exposure to TPA (1 ug/ml) and mitomycin C (0.03 ug/ml) or UV light. In addition to its weak SCE-inducing effects in CHO cells, TPA alone caused a slight delay in cell cycle progression and markedly enhanced the cell cycle delay induced by BP. The SCE-inducing capacity of TPA in these cells was unrelated to its toxicity, because TPA was not toxic at the doses used. It also seemed to be unrelated to excision repair capacity, because TPA caused similar increases in SCE frequency in cells which were proficient (GM637), mildly deficient (CHO), and severely deficient (XP12RO) in excision repair. The significance of SCE induction and its relationship to other biologic effects of TPA remain unknown. (40 Refs)

76. 12-O-TETRADECANOYL-PHORBOL-13-ACETATE AND ITS RELATIONSHIP TO SCE INDUCTION IN SYRIAN AND CHINESE HAMSTER CELLS.

Popescu NC, Amsbaugh SC, Larramendy ML, DiPaolo JA
Lab. Biology, Building 37, Room 2A19, NCI, Bethesda, MD, 20205
Environ Mutagen; 4(1):73-81 1982

Alteration of cell culture conditions, 12-O-tetradecanoylphorbol-13-acetate (TPA), or 5-bromodeoxyuridine (Burd) concentrations, or temporal relation between UV or x-irradiation and TPA had no effect on sister chromatid exchange (SCE) induction of Syrian hamster embryo cells (HEC). SCE frequencies were determined on exponentially growing and stationary HEC cultures incubated with different Burd concentrations for one or two replicative cycles. TPA (0.01-2.0 ug/ml medium) was ineffective in inducing SCE in HEC cultures cultivated in medium supplemented with heat-inactivated serum, independent of Burd concentration (1.0-10.0 ug/ml) and the number of cell divisions post treatment. In a similar study, TPA did not increase SCE frequency in V79-4 Chinese hamster cells in heat-inactivated or noninactivated serum. TPA has no effect on UV or x-irradiation induced SCE. X-irradiation (200 R) causes an increase of only 1 SCE/cell over the control value, while UV (1.5 and 3.0 J/square meter) causes an increase of 10 and 24 SCE per metaphase. TPA addition either immediately or 48 hr after UV or x-irradiation did not alter SCE frequency obtained by irradiation alone. Although SCE induction may be important for the induction of transformation by environmental agents, the enhancement of transformation frequency caused by TPA occurs without further DNA alterations involved in SCE formation. (21 Refs)

77. CORRELATION BETWEEN D REGION STRUCTURE AND ANTIGEN-BINDING SPECIFICITY: EVIDENCES FROM THE COMPARISON OF CLOSELY RELATED IMMUNOGLOBULIN VH SEQUENCES.

Auffray C, Sikorav JL, Ollo R, Rougeon F
Unite de Genetique et Biochimie de Developpement, E.R.A. CNRS 851, Institut Pasteur, 75724 Paris Cedex 15, France
Ann Immunol (Paris); 132(1):77-88 1981

Complementary DNA (cDNA) clones containing VH sequences of three myeloma proteins, of which two (UPC10 and ABE48) bind 2,6-levan, and one (MOPC173) has an unknown specificity, were constructed and sequenced. cDNA clones were compared to four sequences of anti-galactan myeloma proteins belonging to NIH BALB/c plasmacytoma. Starting with partially purified alpha heavy chain messenger RNA (mRNA) from ABE48 myeloma and gamma2a heavy chain mRNA from MOPC173 myeloma, collections of recombinant plasmids were constructed. Plasmids palpha48-16 and pG2a-173-27, which carry the most important part of the corresponding VH sequences were isolated using 650-base pair PstI fragment of pG2a-10-21 containing the VH sequence. Nucleotide sequences are presented. As a result of the cloning procedure, the sequence corresponding to the 5' end of the mRNA is not present in pG2a-173-27, which begins at the codon 5 of the variable region of the heavy chain (VH region), and in palpha48-16, which begins at codon 32 of the VH region. The amino-acid se quences presented were derived from the nucleotide sequences. The MOPC173 sequence agrees with a previ-

ously reported except for the exchange Asn/Asp at codons 52-54 and 73-74. Results confirm the strong correlation existing between D-region structure and antigen-binding specificity. (29 Refs)

78. RECOMBINATION AND MUTAGENESIS IN RAD6 MUTANTS OF SACCHAROMYCES CEREVISIAE: EVIDENCE FOR MULTIPLE FUNCTIONS OF THE RAD6 GENE.

Montelone BA, Prakash S, Prakash L
(c/o L. Prakash) Dept. Radiation Biology and Biophysics, Sch. Medicine, Univ. Rochester, Rochester, NY, 14642
MGG; 184(3):410-415 1981

The rad6-1 and rad6-3 mutants are highly UV sensitive and show an increase in spontaneous and UV induced mitotic heteroallelic recombination in diploids. Both rad6 mutants are proficient in spontaneous and UV induced unequal sister chromatid recombination in the reiterated ribosomal DNA sequence and are deficient in UV induced mutagenesis. In contrast to the above effects where both mutants appear similar, rad6-1 mutants are deficient in sporulation and meiotic recombination whereas rad6-3 mutants are proficient. The differential effects of these mutations indicate that the RAD6 gene is multifunctional. The possible role of the RAD6 gene in error prone excision repair of UV damage during the G1 phase of the cell cycle in addition to its role in postreplication repair is discussed. (Author abstract) (41 Refs)

79. DNA PROFILE AND TUMOUR PROGRESSION IN PATIENTS WITH SUPERFICIAL BLADDER TUMOURS.

Gustafson H, Tribukait B, Esposti P
Dept. Urology, Karolinska Hosp., Stockholm, Sweden
Urol Res; 10(1):13-18 1982

Two hundred-twenty nine cases of surgically treated superficial bladder tumors Grade 1-2, category Ta or T1, were followed clinically and by flow cytofluorometry (FCM). The mean follow-up time for continuous FCM was 2.6 yr. Three different types of DNA patterns were found in Grade 2 tumors: diploid DNA pattern; tetraploid DNA pattern; and non-tetraploid types with hypo- and hypertetraploid patterns. One hundred-seventy five cases were diploid and 54 were aneuploid. Tumor progression was not seen in the 175 cases with repeatedly diploid DNA pattern. In the cases with tumor progression, the degree of ploidy could be related to the frequency of progression: 50% of the cases with triploid-hypotetraploid DNA pattern showed progression, while 10% with tetraploid DNA showed progression. FCM analysis in 33 cases of recurring tumors revealed that the degree of ploidy and the proportion of S-phase cells was fairly constant. The results indicate that the DNA profile can characterize a bladder tumor by its degree of ploidy and proliferation, and may be significant in predicting progression in cases of well and moderately well differentiated superficial bladder carcinoma. (11 Refs)

80. DNA DEPENDENCE AND INHIBITION BY NOVOBIOCIN AND COUMERMYCIN OF THE NUCLEOLAR ADENOSINE TRIPHOSPHATASE (ATPASE) OF HUMAN FIBROBLASTS.

Fox N, Studzinski GP
Dept. Pathology, CMDNJ-New Jersey Medical Sch., Newark, NJ, 07103
J Histochem Cytochem; 30(4):364-370 1982

We have recently demonstrated by electron microscopic cytochemical methods that unfixed human fibroblasts exhibit intense Mg++ dependent adenosine triphosphatase (nATPase) activity in circumscribed areas of the cell nucleoli. The nATPase was specific for ATP and dATP and was inhibited by other ribonucleoside triphosphates. Its intranucleolar localization relative to nucleolar chromatin, and segregation into nucleolar zones after actinomycin treatment of the cells, suggested that the reaction took place in fibrillar centers. This ATPase has now been further characterized by electron microscopic cytochemistry. It was determined that short fixation permitted retention of most of the ATPase activity, and that the enzyme was active at high ionic strength (up to 400 mM KCl), but that the enzyme activity was very sensitive to elevated temperatures. DNA dependence of the enzyme was shown by inhibition of the reaction by DNase pretreatment in parallel with the removal of DNA from the cell, while pretreatment with RNase had no significant effect. The nATPase activity was also selectively inhibited by treatment of the cells with antagonists of the B subunit of DNA gyrase, novobiocin, and coumermycin, but not by nalidixic or oxolinic acids, which interfere with the A subunit of gyrase. Inhibitors of RNA synthesis, actinomycin D and aminonucleoside of puromycin, potentiate rather than inhibit nATPase reaction. The results suggest that nATPase functions to alter the degree of supercoiling or catenation of nucleolar organizer DNA, and is in reality a DNA topoisomerase that hydrolyzes ATP during its action. (Author abstract) (36 Refs)

81. SIMILARITIES BETWEEN MOUSE AND RAT-LIVER MICROSOMAL CYTOCHROMES P-450 INDUCED BY 3-METHYLCHOLANTHRENE: EVIDENCE FROM CATALYTIC, IMMUNOLOGIC, AND RECOMBINANT DNA STUDIES.

Chen YT, Lang MA, Jensen NM, Negishi M, Tukey RH, Sidransky E, Guenther TM, Nebert DW
Development Pharmacology Branch, Natl. Inst. Child Health and Human Development, NIH, 9000 Rockville Pike, Bethesda, MD, 20205
Eur J Biochem; 122(2):361-368 1982

Anti(P1-450) and anti(P-448) are two antibodies developed against distinctly different forms of 3-methyl-cholanthrene-induced cytochrome P-450 in C57BL/6N mouse liver microsomes. The effects of these antibodies on Long-Evans and Sprague-Dawley rat liver microsomes and rat 'minimal deviation' hepatoma 7777 microsomes were studied. Rat microsomal aryl hydrocarbon hydroxylase activity, induced by polycyclic aromatic compounds, is preferentially inhibited by anti-(P1-450) more so than anti-(P-448). Polycyclic-aromatic-induced rat liver microsomal acetanilide 4-hydroxylase activity is preferentially inhibited by anti-(P-448) more so than anti-(P-450). Anti-(P-448) precipitates a 55-kDa moiety in control, phenobarbital-treated, or polycyclic-aromatic-induced rat liver microsomes. A heterogeneity was found amongst individual Morris 'minimal deviation' hepatomas 7777, indicating the 3-methylcholanthrene-induced aryl hydrocarbon hydroxylase and acetanilide 4-hydroxylase activities must represent different forms of rat P-450. Clone 46 DNA, a portion of the mouse cloned P1-450 structural gene, hybridizes to one gene (or a small number of genes) in rat liver and to 3-methylcholanthrene-induced rat 23-S P1-450 mRNA. As has already been shown in the mouse, rat liver P1-450 induction is under transcriptional control, and there is no evidence for gene amplification or some gross form of DNA rearrangement. These data demonstrate striking similarities between rat and mouse for the P1-450 structural gene, the P1-450 mRNA, the P1-450 protein and the P-448 protein. Polycyclic-aromatic-induced P1-450 and its association with polycyclic-aromatic-induced aryl hydrocar-

bon hydroxylase activity, therefore does not appear to be closely correlated with polycyclic-aromatic-induced P-448 in either rat or mouse. (Author abstract) (46 Refs)

* U. S. GOVERNMENT PRINTING OFFICE : 1982—361-547/123

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JAMES K. SELKIRK Oak Ridge National Laboratories	*Metabolic Activation and Macromolecular Binding of Chemical Carcinogens*	#VK02 51 min.
PHILIP C. HANAWALT Stanford University	*Cellular Responses to Damaged DNA*	#VK03 54 min.
CHARLES HEIDELBERGER University of Southern California	*Mutagenesis and Oncogenic Transformation of Mouse Embryo Cells*	#VK04 54 min.
FRED RAPP Milton S. Hershey Medical Center	*The Interaction of Herpesviruses and Host Cells Leading to Latency or Transformation*	#VK05 52 min.
	Panel Discussion	#VK06 31 min.

MECHANISMS OF METASTASIS

ISAIAH FIDLER Frederick Cancer Research Center	*The Heterogeneous Nature of Cancer Metastasis and Implications for Therapy*	#VB01 47 min.
GEORGE POSTE Smith, Kline and French Laboratories	*Phenotypic Analysis of Metastatic Cell Populations*	#VB02 42 min.
LANCE LIOTTA National Cancer Institute	*Tumor Cell Invasion of Basement Membranes and Extracellular Matrices*	#VB03 33 min.
EVERETT SUGARBAKER Miami Cancer Institute	*Some Clinical Characteristics of Metastasis in Man*	#VB04 41 min.

AUTOMATED CYTOLOGY

Part One: Principles and Techniques	#VB05 56 min.
Part Two: Biological Applications	#VB06 52 min.

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MECHANISMS OF DNA REPLICATION

1. ADENOVIRAL PROTEIN-PRIMED INITIATION OF DNA CHAINS IN VITRO.

Ikeda JE, Enomoto T, Hurwitz J
Dept. Molecular Genetics, Inst. Plant Virus Res., Tsukuba Science-City, Yatabe, Ibaraki 305, Japan
Proc Natl Acad Sci USA; 79(8,part1):2442-2446 1982

The initiation of DNA chains by the 80-kilodalton form of the adenovirus terminal protein has been studied. This protein, which can be covalently linked to dCMP, is isolated complexed to a 140-kilodalton protein possessing DNA polymerase activity. In the presence of adenovirus DNA-protein, the formation of the 80-kilodalton protein-dCMP complex requires the addition of ATP and nuclear extract from uninfected cells

HE 20.3172/2:

in addition to Mg++ and dCTP. When single-stranded DNA is used in place of the adenovirus DNA-protein, the formation of the 80-kilodalton protein-dCMP complex occurs in the absence of ATP and nuclear extract. In the presence of the four dNTPs, the complex yields DNA chains of various sizes between 100 and 300 nucleotides. The products formed with bacteriophage phiX174 single-stranded circular DNA as the template are site specific, predominantly derived from the sequences between nucleotides 2363 and 2977 and between nucleotides 3760 and 4206. These small DNA chains are blocked at their 5' ends with the 80-kilodalton protein but possess free 3'-OH ends that are susceptible to degradation by exonuclease III and can be elongated to replicative form II products with DNA polymerase I of Escherichia coli or eukaryotic DNA polymerase beta preparations. A protein priming model explaining the different requirements for initiation with adenovirus DNA-protein and with phiX174 DNA is presented. (Author abstract) (20 Refs)

2. DETECTION OF A 10 KB DNA REPLICATION INTERMEDIATE IN HUMAN MELANOMA CELLS.

Lonn U
Dept. Histology, Karolinska Institutet and Radiumhemmet, Karolinska Hosp., S-10401 Stockholm, Sweden
Chromosoma; 84(5):663-673 1982

DNA replication in human melanoma cells is investigated by lysing the cells in dilute alkali. This lysis condition results in the release from parental DNA of the single-stranded DNA fragments located in active replicating units. The size of the released DNA should theoretically range from that Of Okazaki-fragments up to that of the entire replication unit. However, the results showed that the released DNA replication intermediates which are detected range in the size between Okazaki-fragments up to 10 kb DNA fragments. The 10 kb DNA fragments show a discrete appearance in agarose gel electrophoresis. Moreover the kinetic results indicate that the ligation of the 10 kb DNA fragments to form high mol wt DNA is a late step. A prerequisite for the release of this DNA fragment as a discrete population is that there are gaps in the continuity of the newly synthesized DNA spaced roughly 10 kb away from each other. (Author abstract) (13 Refs)

3. ARREST OF CHAIN GROWTH OF REPLICON-SIZED INTERMEDIATES BY APHIDICOLIN DURING RAT FIBROBLAST CELL CHROMOSOME REPLICATION.

Yagura T, Kozu T, Seno T
Dept. Immunology and Virology, Saitama Cancer Center Res. Inst., 818 Komuro, Ina-machi, Saitama 362, Japan
Eur J Biochem; 123(1):15-21 1982

The effect of aphidicoiin, a specific inhibitor of DNA polymerase alpha, on size maturation of nascent DNA intermediates was studied in cultured rat fibroblast cells. Results provided the first evidence of DNA synthesis associated with merging of intermediates of larger than replicon size. Aphidicolin at a concentration (1.4 ug/ml) causing 90-95% inhibition of [3H]thymidine incorporation, resulted in accumulation of intermediates of nearly the same size as the replicon (2-5 x 10(7) Da); although the synthesis of short nascent fragments (referred to as Okazaki fragments) continued in the presence of aphidicolin, the rate of their elongation to the replicon size was greatly decreased. On removal of aphidicolin, these accumulated intermediates merged into high-mol wt DNA. This merging of the intermediates was associated with DNA synthesis in gaps between adjacent intermediates, as revealed by photolysis of bromodeoxyuridine-DNA leader with long-wave ultraviolet light; when the cells had been pulse-labeled for 5 min with bromodeoxyuridine immediately after removal of the drug, the large DNA arising from aphidicolin-arrested intermediates was cut into fragments of the original size by long-wave ultraviolet light irradiation. The arrest of chain elongation at the replicon-size by aphidicolin might be due to inhibition of this DNA synthesis in gaps, because aphidicolin did not cause degradation of nascent DNAs. (Author abstract) (37 Refs)

4. EFFECTS OF ALKYLATING AGENTS ON THE DNA REPLICATION OF CULTURED YOSHIDA SARCOMA CELLS.

Van Der Steen J, Van Wichen BH, Benckhuysen C
Div. Experimental Chemotherapy and Radiotherapy, Antoni Van Leeuwenhoek-Huis, The Netherlands Cancer Inst., Amsterdam, The Netherlands
Chem Biol Interact; 39(2):191-204 1982

Logarithmically growing Yoshida sarcoma cells were treated for 1 hr with low (2 decades cell kill) or high (more than 6 decades cell kill) doses of alkylating agent. Pulse and chase labeled DNA from treated cells were studied by alkaline sucrose gradient centrifugation. Nitrogen mustard (HN-2), 4-hydroperoxycyclophosphamide (CY-OOH), melphalan (L-PAM) and chlorambucil (CA) had no effect on the elongation rate of newly replicated DNA, both at low and high doses, although per cell the rate of DNA synthesis declined as inferred from the rates of [3H]thymidine incorporation compared to the increase in numbers of S phase cells in the treated populations. It is concluded that these drugs act specifically on the initiation step of the DNA replication, leaving chain elongation undisturbed. At low doses the chemically related sulfur mustard (SM) had also no effect on the maturation of new DNA but at high doses a decreased elongation rate was observed. A transient inhibition of chain growth was observed following treatment with a low dose of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). In contrast, the intercalating agent adriamycin showed a severe but delayed effect resulting in an almost complete block of the maturation. (Author abstract) (24 Refs)

5. NUCLEOSOME SEGREGATION AT A DEFINED MAMMALIAN CHROMOSOMAL SITE.

Roufa DJ, Marchionni MA
Div. Biology, Kansas State Univ., Manhattan, KS, 66506
Proc Natl Acad Sci USA; 79(6,part1):1810-1814 1982

When animal cells replicate chromatin under conditions precluding new histone biosynthesis, half of the daughter DNAs are devoid of nucleosomes and are sensitive to staphylococcal nuclease. DNA sequences resistant to nuclease are associated with preexisting nucleosomes, which redistribute to progeny DNA duplexes during replication. We labeled newly replicated DNA sequences in a simian virus 40 (SV40)-transformed Chinese hamster cell clone with 5-bromodeoxyuridine (BrdUrd) in the presence and absence of a protein biosynthesis inhibitor, emetine. We resolved single-stranded BrdUrd- and dT-DNA sequences protected from nuclease digestion by nucleosomes and determined from which strands of the integrated viral DNA parental template (dT) and newly replicated progeny (BrdUrd) sequences were derived. Because we knew that the cell clone studied contained all of its integrated SV40 DNA at a single chromosomal site, we were able to determine that preexisting nucleosomes segregated to only one of the two daughter duplexes containing the integrated viral sequence. Additionally, in the presence of emetine, the integrated viral origin of replication, ORIsv, appeared not to function as a chromosomal

replication origin, perhaps reflecting the drug's effect on synthesis of SV40 large tumor antigen. (Author abstract) (20 Refs)

6. THE ORDER OF DNA REPLICATION IN MAMMALIAN CELLS. A MODIFIED DENSITY LABELLING PROCEDURE FOR ISOLATING SEQUENCES REPLICATED AT SPECIFIC TIMES.

Grady LJ, Sanders ML
Div. Lab. and Res., New York State Dept. Health, Albany, NY, 12201
Exp Cell Res; 138(2):480-485 1982

These experiments were directed towards establishing a procedure for isolation of the DNA sequences replicated at different times in S phase for further study. Primary considerations were thus for a method that gave fairly large quantities of cells as well synchronized in S phase as possible. The use of Chinese hamster ovary (CHO) cells synchronized by isoleucine starvation followed by exposure to hydroxyurea (1) seemed to be most promising and was explored in detail. During the course of this work, a labeling scheme was developed that involved exposure to BrdUrd during the same time window in two sequential S phases. Only those regions of the genome replicated in the same interval in each S period contained BrdUrd in both strands and these were readily isolated in CsCl gradients. As applied in the present experiments, this labeling procedure showed no untoward effects on cell-cycle traverse and gave DNA distributions in density gradients that closely approximated those that would be expected for the conditions employed. (Author abstract) (11 Refs)

EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

7. SELECTIVE INHIBITION OF IN VITRO SYNTHESIS OF CANCER DNA BY ALKALOIDS OF BETA-CARBOLINE CLASS.

Beljanski M, Beljanski MS
Laboratoire de Pharmacodynamie, Faculte de Pharmacie, F-92290 Chatenay-Malabry, France
Exp Cell Biol; 50(2):79-87 1982

The high template in vitro activity of native DNA from cancerous mammalian and plant tissues, compared to DNA from healthy tissues, enabled us to select substances which selectively inhibit cancer DNA synthesis. Among them, alstonine, serpentine, sempervirine and flavopereirine, all alkaloids which belong to the beta-carboline class, distinguish cancer DNA from healthy tissue DNA and inhibit DNA in vitro synthesis when native DNA from different cancerous tissues or cells is used as template. They have practically no effect on DNA from healthy tissues. The inhibitory effect of alkaloids is due to their capacity to form on 'alkaloid-cancer DNA' complex which has been characterized by use of the Sephadex column. Evidence is presented showing that these alkaloids inhibit the initiation of DNA synthesis but not chain elongation. The stimulating action caused by carcinogens during cancer DNA in vitro synthesis may be prevented and reversed by alkaloids. Furthermore, the stimulating action of steroids during in vitro synthesis of hormone target tissue DNA might be neutralized by alkaloids. However, at relatively high doses, steroids reversibly compete with alkaloids for binding sites on breast cancer DNA. This is not observed with DNA from nonhormone target tissues. (Author abstract) (19 Refs)

8. STIMULATION OF LIVER CELL DNA SYNTHESIS BY ONCOMODULIN, AN MW 11,500 CALCIUM-BINDING PROTEIN FROM HEPATOMA.

Boynton AL, MacManus JP, Whitfield JF
Animal and Cell Physiology Group, Div. Biological Sciences, Natl. Res. Council of Canada, Ottawa, K1A OR6, Canada
Exp Cell Res; 138(2):454-458 1982

Raising the calcium concentration, or adding the tumor-specific calcium-binding protein oncomodulin (but not a similar, calcium-binding protein such as skeletal muscle parvalbumin) stimulated DNA synthesis in non-neoplastic T51B rat liver cells, whose DNA-synthetic activity had been reduced by incubation in medium containing 0.02 mM calcium instead of the usual 1.8 mM calcium. A calcium:oncomodulin complex was probably the actual stimulator, because oncomodulin action was blocked by further reducing the ionic calcium concentration in the already calcium-deficient medium with EGTA. Oncomodulin was also able to stimulate DNA synthesis in T51B cell cultures, whose response to calcium addition had been blocked by trifluoperazine. (Author abstract) (32 Refs)

9. SELECTIVE INHIBITION OF DNA SYNTHESIS BY A PROTEIN RELEASED FROM SPLEEN CELLS.

Miyata S, Kihara HK
Lab. Res. Biosynthesis and Metabolism, Sch. Medicine, Keio Univ., Tokyo 160, Japan
J Cell Physiol; 110(3):315-317 1982

A protein isolated from the medium of cultured mouse embryo spleen cells was found both to bind to single-stranded DNA and to inhibit incorporation of H3-thymidine into DNA of certain cells. While DNa synthesis was strongly inhibited by this protein in HeLa, L, HEp2 cells, Ehrlich cells and primary mouse embryo cells were relatively refractory to its effects. (16 Refs)

10. INHIBITION OF DNA SYNTHESIS AND ALTERATION TO DNA STRUCTURE BY THE PHENACETIN ANALOG P-AMINOPHENOL.

Hayward NK, Lavin MF, Craswell PW
(c/o Lavin) Dept. Biochemistry, Univ. Queensland, Brisbane 4067, Australia
Biochem Pharmacol; 31(7):1425-1429 1982

p-Aminophenol a structural analog and minor metabolite of phenacetin has previously been shown to be a potent nephrotoxic agent. In this report we have shown that p-aminophenol has a marked effect on DNA function and structure. DNA synthesis was inhibited in a dose-dependent manner in human lymphoblastoid cells after exposure to p-aminophenol. Results suggest that DNA synthesis is inhibited by the action of p-aminophenol on DNA structure. At low concentrations of p-aminophenol a reduction in the degree of supercoiling of cellular DNA is observed, as determined by sedimentation under neutral conditions. However at higher concentrations an increase in sedimentation of nucleoids (supercoiled molecules) is obtained which is indicative of an increased level of supercoiling or a more compact structural form of DNA due to folding or aggregation. The number of single strand breaks in DNA, when determined by sedimentation in alkaline sucrose gradients, increases with increasing dose of p-aminophenol. The increase in strand breakage observed at lower concentrations of p-aminophenol agrees with the reduced sedimentation rate obtained under neutral conditions. At higher concentrations of p-aminophenol the extent of breakage of DNA increases under

alkaline conditions but an increase in sedimentation occurs under neutral conditions. (Author abstract) (23 Refs)

11. GENE CONTROLLED NEGATIVE REGULATION OF DNA SYNTHESIS IN ERYTHROPOIETIC PROGENITOR CELLS.

Axelrad A, Croizat H, Eskinazi D, Stewart S, Vaithilingam D, Van der Gaag H
Div. histology, Dept. Anatomy, Univ. Toronto, Toronto, Ontario M5S 1A8, Canada
J Cell Physiol; Suppl1:165-173 1982

Negative regulation of DNA synthesis in early erythropoietic progenitor cells (BFU-E) and its relation to Friend polycythemia virus (FV) are discussed. Evidence for the negative regulatory activity in B6 mice marrow cells is presented. Results indicate that the activity is under strict control. The activity is detectable at approx the same low concentration at which it is found in B6 marrow, as shown by the rapidity with which it dilutes out in titration curves. The activity appears to be specific for the BFU-E population and is readily reversible without apparent toxicity. No reduction in burst-forming efficiency was observed, and the proportion in DNA synthesis is not reduced by the cells having been in contact with B6 marrow supernatant. Evidence for operation of the negative regulator (NR) on the cell surface is presented. NR is readily removed by a single wash and is immediately effective when added to the cells although its mol wt appears to be high, it is accessible to antibody while associated with the cells, and it functions at 0 C. The NR of BFU-E DNA synthesis was discovered in normal B6 mice during the course of investigations of the mechanism by which the Fv-2rr genotype confers solid resistance against the development of FV-induced polycythemia in adult mice. Effects of B6 NR of BFU-E DNA synthesis on amplification of megakaryocyte colony forming units (CFU-E) by FV and erythropoietin were studied in vitro. Results indicate that inclusion of concentrated B6 marrow supernatant in the liquid cultures entirely prevented the amplification of the CFU-E population brought about by FV and erythropoietin. (28 Refs)

12. CONVERSION OF DNTP TO DNMP DEPENDENT ON DNA SYNTHESIS IN ISOLATED YOSHIDA SARCOMA NUCLEI.

Akiyoshi H
Dept. Enzyme Physiology, Inst. Enzyme Res., Tokushima Univ. Sch. Medicine, Kuramoto-cho, Tokushima 770, Japan
Biochim Biophys Acta; 696(3):332-339 1982

Nuclei isolated from Yoshida sarcoma cells had activity for conversion of dGTP to dGMP dependent on DNA synthesis. The ratio of nucleotide generation/generation + incorporation was 0.4 +-0.1, indicating that approx 40% of the incorporated dGMP was excised. Two lines of evidence indicated the dependence of this activity on DNA synthesis. (1) The activity was observed only in the presence of ATP, which is essential for nuclear DNA synthesis. (2) Inhibitors of DNA synthesis, such as N-ethylmaleimide, aphidicolin, spermine and KCl, also inhibited ATP- or DNA synthesis-dependent dGMP generation. Although nuclei contain nucleoside triphosphatase (N-nucleotidase), this enzyme was not involved appreciably in DNA synthesis-dependent dGMP generation. The reason for this was explained by the following findings. (a) Inhibitors did not decrease dGMP production in the complete absence of DNA synthesis. (b) Inhibitors did not inactivate N-nucleotidase to the same degree as they inhibited DNA synthesis-dependent dGMP generation. (c) Addition of ATP reduced dGTP hydrolysis catalyzed by N-nucleotidase. (d) GDP had no appreciable effect on DNA synthesis-dependent dGMP generation, but had a diluting effect on dGMP production catalyzed by N-nucleotidase. These results show that the pathway of dGMP generation in isolated nuclei was switched on addition of ATP from a N-nucleotidase-catalyzed one to a DNA polymerase-exonuclease-catalyzed one. (Author abstract) (38 Refs)

13. THE EFFECTS OF METHYLATED THYMIDINES UPON CULTURES OF V79 CELLS AND THE MECHANISM OF INCORPORATION OF O(4)-METHYLTHYMIDINE INTO THEIR DNA.

Brennand J, Saffhill R, Fox M
Paterson Lab., Christie Hosp. and Holt Radium Inst., Manchester M20 9BX, England
Carcinogenesis; 3(2):219-222 1982

High concentrations of O2-methylthymidine, 3-methylthymidine and O4-methylthymidine (O4-MedT) have little effect on the colony forming ability of cultured Chinese hamster (V79) cells. However, the methylated derivatives enhance the toxic effects of thymidine (dT) at concentrations at which the modified nucleosides alone show no toxicity. O4-MedT was the only modified nucleoside which is incorporated into V79 cell DNA at a detectable level. In the presence of added dT there is an increase in the amount of O4-MedT incorporated. O4-MedT did not induce mutation, either forward or reverse, in V79 cells. The results indicate that O4-MedT is incorporated into DNA as an analogue of deoxycytidine and once incorporated behaves as cytidine during DNA synthesis, in contrast to O4-MedT produced in cellular DNA by methylating carcinogens which is miscoding and potentially mutagenic. (Author abstract) (19 Refs)

14. INDUCTION OF SISTER CHROMATID EXCHANGES AND INHIBITION OF CELLULAR PROLIFERATION IN VITRO. I. CAFFEINE.

Guglielmi GE, Vogt TF, Tice RR
(c/o Tice) Medical Dept., Brookhave Natl. Lab., Upton, NY, 11973
Environ Mutagen; 4(2):191-200 1982

The ability of caffeine to induce sister-chromatid exchanges (SCEs) and to inhibit cellular proliferation of human lymphocytes in vitro was examined. Caffeine (0.10-4.10 milli(m)M) inhibited cellular proliferation in phytohemagglutinin-stimulated lymphocytes exposed continuously, but not in those receiving an acute 4-hr exposure. Administration of caffeine throughout the culture period induced a significant dose-dependent increase in SCEs, but exposure for 4 hr prior to the DNA synthetic period did not change SCE frequency. Using a linear regression curve derived from least squares analysis of the semilogarithmic plot of rate of SCE induction, the caffeine concentration capable of doubling the control SCE frequency was calculated as 2.4 mM. SCE induction rate in each of three bromodeoxyuridine replication cycles was the same. The mechanism for the results reported here is probably inhibition of DNA synthesis. Inhibition of DNA synthesis by caffeine may involve purine metabolism, transport, and salvage utilization in a direct or an indirect manner. The non-linearity of the SCE dose response curve for caffeine is in contrast to the typical linear graph for other well-known mutagenic/carcinogenic agents, suggesting that there is more than one mechanism of SCE induction. (52 Refs)

15. FLOW CYTOMETRIC CHARACTERIZATION OF THE RESPONSE OF FANCONI'S ANEMIA CELLS TO MITOMYCIN C TREATMENT.

Kaiser TN, Lojewski A, Dougherty C, Juergens L, Sahar E, Latt SA
Dept. Pediatrics, Harvard Medical Sch., Boston, MA, 02115
Cytometry; 2(5):291-297 1982

Flow cytometric characterization of the response of Fanconi's anemia (FA) cells to mitomycin C (MMC) treatment was performed on dermal fibroblasts that had been passaged in culture 3-14 times and obtained from eight patients with FA. The patients were diagnosed by classical criteria as well as by manifestation of cytogenetic abnormalities following exposure of peripheral lymphocytes and/or fibroblasts to MMC. Fibroblasts from four parents of two patients, assumed to be obligate heterozygotes, and from seven normal individuals were also included into the study. In order to select MMC concentrations that might distort DNA flow histograms without completely inhibiting cell cycle progression, 3H-deoxythymidine incorporation into DNA at various intervals following MMC treatment was measured. The best discrimination between cells from controls and from patients occurred 24-48 hr after a 2-hr exposure to 0.05-0.10 ug/ml MMC. Av DNA synthesis rates were depressed from approx 40-95%. Inhibition of DNA synthesis was slightly greater in cells sorted from the second half of S phase. The major effect of MMC on DNA flow histograms was an increase in the fraction of cells in the G2 + M Gaussian peak. Additional experiments were performed using ethylmethanesulfonate (EMS) to characterize the specificity of the effect of MMC on FA cells. EMS, at concentrations sufficient to more than double the sister chromatid exchange frequency in normal cells, did not effect a differential accumulation of FA cells in late S or G2 + M phase. (52 Refs)

16. COMPLEMENTATION OF THE DEFECTS IN DNA SYNTHESIS IN IRRADIATED AND UNIRRADIATED ATAXIA-TELANGIECTASIA CELLS.

Murnane JP, Painter RB
Lab. Radiology and Environmental Health, Univ. California, San Francisco, CA, 94143
Proc Natl Acad Sci USA; 79(6,part1):1960-1963 1982

Mutant subtypes in the human genetic disease ataxia-telangiectasia (A-T) were classified by means of an assay system that monitors complementation of defects in DNA synthesis. Anomalies in DNA synthesis have been observed previously in A-T cells, both in their failure to inhibit DNA synthesis immediately after exposure to ionizing radiation and in their prolonged S phase. Polyethylene glycol-mediated cell fusion and autoradiography were combined with selective identification of different A-T cell populations by fluorescent colored microspheres to determine complementation capabilities of various A-T cell combinations. Five complementation groups were identified by both a 30-40% increase in the rate of DNA synthesis in unirradiated heterokaryons and the appearance of normal inhibition of DNA synthesis after x-irradiation of heterokaryons. The correlation observed between these phenomena suggests that the defects in A-T cells involve problems in initiation of DNA synthesis. (Author abstract) (20 Refs)

17. GENETIC HETEROGENEITY IN ATAXIA-TELANGIECTASIA STUDIED BY CELL FUSION.

Jaspers NG, Bootsma D
Lab. Cell Biology and Genetics, Erasmus Univ. Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands
Proc Natl Acad Sci USA; 79(8,part1):2641-2644 1982

The effect of x-rays on the rate of semiconservative DNA replication was investigated by autoradiography in single cells obtained from normal individuals and from patients having ataxia-telangiectasia (AT). In the five AT cell strains studied, the rate of DNA synthesis was inhibited to a lesser extent than in two normal cell strains. By using this abnormal regulation of DNA replication in AT cells as a marker, an experimental procedure was developed that allowed genetic complementation analysis of AT. After Sendai virus-induced fusion of AT cells, the grains were counted over binucleate cells with both nuclei in S phase. In some cases, the inhibition of DNA synthesis caused by x-rays in the heterodikaryons was more pronounced than that in the parental homodikaryons and was comparable to that in normal binucleate cells, indicating complementation. By using this approach, the five AT cell strains that were investigated could be assigned to three complementation groups. The data suggest that extensive genetic heterogeneity exists in AT. (Author abstract) (15 Refs)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

18. CAFFEINE INHIBITS DNA POLYMERASE I FROM ESCHERICHIA COLI: STUDIES IN VITRO.

Balachandran R, Srinivasan A
Lab. Cellular and Molecular Biology, NCI, Bethesda, MD, 20205
Carcinogenesis; 3(2):151-153 1982

Caffeine inhibits the activity of DNA polymerase I (E coli) and its proteolytic large fragment in in vitro DNA replication system. DNA polymerase from Micrococcus luteus is also equally inhibited by caffeine. The extent of inhibition was more with the activated adenovirus, T4 and calf thymus DNA than with synthetic DNA template-primers. Results obtained from time-course studies indicated that caffeine inhibition reached maximum by 30 min of incubation. Enzyme kinetic studies showed that inhibition was competitive with respect to DNA template. (Author abstract) (19 Refs)

19. COMPARISON OF THE ACTIONS OF 9-BETA-D-ARABINOFURANOSYL-2-FLUOROADENINE AND 9-BETA-D-ARABINOFURANOSYLADENINE ON TARGET ENZYMES FROM MOUSE TUMOR CELLS.

White EL, Shaddix SC, Brockman RW, Bennett LL
(c/o Bennett) Kettering-Meyer Lab., Southern Res. Inst., P.O. Box 3307-A, 2000 Ninth Ave. South, Birmingham, AL, 35255
Cancer Res; 42(6):2260-2264 1982

The compound 9-beta-D-Arabinofuranosyl-2-fluoroadenine (2-F-ara-A), a derivative of 9-beta-D-arabinofuranosyladenine (ara-A) that is resistant to deamination, selectively inhibits DNA synthesis and has activity against mouse leukemia L1210 comparable to that of ara-A plus the adenosine deaminase inhibitor, 2'-deoxycoformycin. To determine if these two nucleosides have similar modes of action, comparisons were made of their effects and those of their triphosphates on enzymes known to be inhibited by ara-A or 9-beta-D-arabinofuranosyladenine 5'-triphosphate. 9-beta-D-Arabinofuranosyl-2-fluoroadenine 5'-triphosphate was more effective than 9-beta-D-arabinofuranosyladenine 5'-triphosphate in inhibiting the reduction of adenosine 5'-diphosphate and cytidine 5'-diphosphate by ribonucleotide reductase from HEp-2 cells or L1210 cells. DNA polymerase alpha from L1210 cells was equally sensitive to 9-beta-D-arabinofuranosyl-2-fluoroadenine

5'-triphosphate and 9-beta-D-arabinofuranosyladenine 5'-triphosphate, and DNA polymerase beta from L1210 cells was much less sensitive to both triphosphates. S-adenosylhomocysteine hydrolase from L1210 cells was inactivated by 2-F-ara-A and ara-A, but higher concentrations of the fluoro derivative were required. These results are consistent with 2-F-ara-A and ara-A inhibition of DNA synthesis by inhibition of ribonucleotide reductase and DNA polymerase alpha. (Author abstract) (32 Refs)

20. AN OLIGORIBONUCLEOTIDE POLYMERASE FROM SV40-INFECTED CELLS WITH PROPERTIES OF A PRIMASE.

Kaufmann G, Falk HH
Biochemistry Dept., Weizmann Inst. Science, Rehovot 76100, Israel
Nucleic Acids Res; 10(7):2309-2321 1982

A transient decaribonucleotide (iRNA) is covalently linked to nascent eukaryotic DNA chains at their 5' end. Searching for the putative iRNA polymerase (primase), we detected in extracts from SV40-infected cells a DNA-dependent incorporation of UMP residues from UTP into free and DNA linked deca- or similarly sized ribonucleotides. Denatured salmon sperm DNA served as the standard template in this reaction. SV40 FIII DNA was also an effective template, SV40 FII DNA was ineffective while FI yielded mainly free decaribonucleotides. The incorporation depended on the other rNTPs and was resistant to high concentrations of alpha-amanitin and rifamycin AF/013, drugs inhibitory to RNA polymerases I, II and III. The results implicate the decaribonucleotide polymerase in the priming of nascent DNA chains and suggest that the unique size of iRNA is encoded within its primase. (Author abstract) (27 Refs)

21. CELLULAR AND EPSTEIN-BARR VIRUS SPECIFIC DNA POLYMERASES IN VIRUS-PRODUCING BURKITT'S LYMPHOMA CELL LINES.

Allaudeen HS, Rani G
Pharmacology Dept., Yale Univ. Sch. Medicine, New Haven, CT, 06510
Nucleic Acids Res; 10(7):2453-2465 1982

We have determined the levels of cellular DNA polymerases and Epstein-Barr virus specific DNA polymerase in three Burkitt's lymphoma cell lines producing varying amounts of EBV, one of which was induced by 12-O-tetradecanoylphorbol-13-acetate (TPA). There was a proportional increase in the level of EBV-DNA polymerase with an increase in the percent of virus-producing cells. However, there was a reciprocal relationship between the levels of EBV-DNA polymerase and DNA polymerase alpha ie. in cell line containing the highest level of EBV-DNA polymerase, activity of DNA polymerase alpha, but not of DNA polymerase beta, was reduced to an insignificantly low level. TPA does not have any direct effect on activities of either EBV-DNA polymerase or DNA polymerase alpha. EBV-DNA polymerases isolated from cells grown with or without TPA are indistinguishable in their properties such as elution position on phosphocellulose column, molecular weight, mono and divalent cation requirements, pH optimum, and other requirements for optimum activity. Addition of crude extracts of cells grown in presence of TPA to the purified DNA polymerase alpha did not inhibit its activity indicating that the observed loss was not due to any specific inhibitor present in TPA treated cells. Raji, a non-producer cell line, did not contain EBV-DNA polymerase. There was no induction of EBV-DNA polymerase when Raji cells were grown in presence of TPA. The phenomenon of reduction in the levels of DNA polymerase alpha in cells induced to produce EBV may represent a mechanism by which the host DNA replication is shut off following virus infection. (Author abstract) (36 Refs)

22. EVALUATION OF METHODS OF DETECTING TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE IN HUMAN HEMATOLOGIC MALIGNANCIES. COMPARISON OF IMMUNOFLUORESCENCE AND ENZYMATIC ASSAYS.

Cibull ML, Coleman MS, Nelson O, Hutton JJ, Gordon D, Bollum FJ
Dept. Pathology, Univ. Kentucky, Lexington, KY, 40536
Am J Clin Pathol; 77(4):420-423 1982

Terminal transferase (TdT) activity and antigen have been measured in 267 specimens of human bone marrow and peripheral blood by using a biochemical assay for enzymatic activity and an immunofluorescence test for antigen. Oligo p(dA)50 and dGTP were used as reagents in the biochemical assay and either rabbit anti-calf TdT or rabbit anti-human TdT was used as the primary antibody for immunofluorescence. Because both false-positive and false-negative detection of TdT antigen occurs, the biochemical assay of TdT activity is considered the standard against which immunofluorescence assays must be measured. If specimens of cells contained TdT activity, then the immunofluorescence detected antigen in 91% of cases (rabbit anti-calf TdT) and 95% of cases (rabbit anti-human TdT). When no TdT activity was detected, the immunofluorescence test was positive in 7.8% of cases (rabbit anti-calf TdT) and 5.2% of cases (rabbit anti-human TdT). When air-dried slides were shipped by air mail to a distant location before being stained for immunofluorescence, TdT antigen was detected in only 33% of matched pair cases which contained TdT activity. From this study, the authors conclude that with current methodology, immunofluorescence tests for TdT antigen must be carried out on slides prepared in the testing laboratory and that such tests are reliable in more than 90% of cases. However, because a small percentage of results are false positives and false negatives, the authors suggest that if a patient's clinical response is not consistent with the immunofluorescence TdT result, an enzymatic assay for TdT activity be carried out. (Author abstract) (19 Refs)

MECHANISMS OF DNA REPAIR

23. DNA REPAIR AND REPLICATION IN RADIATION-SENSITIVE AND -RESISTANT MOUSE LYMPHOMA CELLS GAMMA-IRRADIATED UNDER AEROBIC AND HYPOXIC CONDITIONS.

Johanson KJ, Wlodek D, Szumiel I
Natl. Defence Res. Inst., S-901 82 Umea, Sweden
Int J Radiat Biol; 41(3):261-270 1982

Repair of DNA damage induced by gamma-rays was studied by the DNA unwinding technique in two strains of murine L5178Y lymphoma cells. These strains differ in sensitivity to low LET radiation by a factor of 1.4. No difference was found in the rate of DNA strand break rejoining during the first 3 hr after irradiation with 2 or 10 Gy of gamma-rays under aerobic conditions either at 0 C or at room temperature. Also, the lesions inflicted by irradiation with 5 Gy of gamma-rays under hypoxia at room temperature were repaired by both strains at the same rate. The final level of unrejoined DNA strand break

was the same in both strains, under all conditions examined, within the limits of resolution of the DNA unwinding technique. The rates of DNA chain elongation were also similar in both strains after irradiation under hypoxic or aerobic conditions. (Author abstract) (33 Refs)

24. THE INHIBITION OF DNA REPAIR BY APHIDICOLIN OR CYTOSINE ARABINOSIDE IN X-IRRADIATED NORMAL AND XERODERMA PIGMENTOSUM FIBROBLASTS.

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Genetics Dept., Univ. Coll., Singleton Park, Swansea SA2 8PP, England
Mutat Res; 94(1):229-234 1982

By incubating x-irradiated human fibroblasts with or without aphidicolin (APC) or cytosine arabinoside (AraC), it was investigated (1) whether DNA polymerase alpha participates in the repair of x-ray-induced DNA damage in normal and xeroderma pigmentosum (XP) cells incapable of excising UV-induced pyrimidine dimers, and (2) whether any responses seen with APC are observed with AraC, a known inhibitor of DNA replication and DNA repair in mammalian cells. Normal fibroblasts and excision-deficient XP fibroblasts were prelabeled with [14C]- and [H3]thymidine, respectively, They were then plated in the same dishes and exposed to a range of x-ray doses prior to incubation with or without either APC or AraC. DNA was then isolated on alkaline sucrose gradients. Profiles of gradients indicated that after doses of 150 or 250 Grays the repair of a fraction of the DNA damage was inhibited in both normal and XP cells by APC or AraC. Incubations with both AraC and APC did not increase the breaks seen with either agent alone. The response to post-irradiation incubation with APC was very similar to that seen with AraC, which was also seen after UV-irradiation. It was suggested that AraC blocks both semi-conservative replication and repair replication in a way similar to that of APC, ie, by blocking DNA polymerase alpha. (12 Refs)

25. ENHANCED RADIOSENSITIVITY AND DEFECTIVE DNA REPAIR IN CULTURED FIBROBLASTS DERIVED FROM ROTHMUND THOMSON SYNDROME PATIENTS.

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Radiation Biology Branch, Health Science Div., Atomic Energy of Canada Ltd., Chalk River, Ontario KOJ 1JO, Canada
Mutat Res; 94(1):213-228 1982

Colony forming ability and DNA repair capacity was measured in cultured fibroblasts from four patients with Rothmund Thomson syndrome (RTS). RTS is an oculocutaneous disorder showing autosomal recessive inheritance. All reported cases have been in caucasians, 70% in females. Ancestry of patients can often be traced to the Swiss or Austrian Alpine areas. The normal strains GM38, GM498 and 265OT was used as parallel controls for the RTS strains. Compared to the normal fibroblast strains, 2/4 RTS strains showed significantly enhanced sensitivity to ionizing radiation delivered under hypoxic conditions. Strain RT1MO also showed enhanced sensitivity when irradiated in air whereas strain RT1SE did not. Hypoxic gamma-ray sensitivity was accompanied by the inefficient removal of a class of DNA damage sensitive to incising activities present in a protein extract of Micrococcus luteus. Defective DNA repair in RTS was confirmed by the detection of reduced levels of repair synthesis. Because the present study was restricted to the gamma-ray responses of RTS strains, it is possible that RTS strains that responded normally to gamma-rays may show defective repair of other carcinogen-induced DNA lesions. (32 Refs)

26. REPAIR DNA SYNTHESIS IN HETEROKARYONS DURING REACTIVATION OF CHICK ERYTHROCYTES FUSED WITH HUMAN DIPLOID FIBROBLASTS OR HELA CELLS.

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Dept. Cell Biology and Genetics, Erasmus Univ. Rotterdam, 3000 DR Rotterdam, The Netherlands
Exp Cell Res; 138(2):469-474 1982

Suppression of unscheduled DNA synthesis (UDS) after exposure to ultraviolet (UV) light in the human nuclei results when diploid human fibroblasts are fused with chick erythrocytes. The suppression is positively correlated with the number of erythrocyte nuclei in the heterokaryons, with a maximal effect at 36 hr after fusion. Evidence is presented that this suppression is due to lowered levels of the enzymes involved in UDS as a result of inhibition of the RNA synthesis by chick components. No suppression of UDS is detected in the human nuclei of the HeLa-chick erythrocyte heterokaryons. In HeLa cells the rate of RNA synthesis is about 10x higher than the rate in the normal diploid fibroblasts, and the relatively small inhibitory influence of the chick components will therefore not lead to a limitation of the enzymes involved in UDS in the HeLa-chick erythrocyte heterokaryons. (Author abstract) (7 Refs)

27. SCINTILLOMETRIC DETERMINATION OF DNA REPAIR IN HUMAN CELL LINES: A CRITICAL APPRAISAL.

Bianchi V, Nuzzo F, Abbondandolo A, Bonatti S, Capelli E, Giorio R, Giulotto E, Mazzaccaro A, Stefanini M, Zaccaro L, Zantedeschi A, Levis AG
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Mutat Res; 93(2):447-463 1982

The ability of a UV- and x-irradiation and a variety of chemical agents to stimulate DNA repair synthesis in human heteroploid EUE cell cultures was tested by a simplified (3H)thymidine-incorporation scintillometric procedure, with the use of hydroxyurea (HU) to suppress DNA replicative synthesis. The percentage residual DNA synthesis, the absolute increase in DNA radioactivity, as the ratio treated/control, the relative increase in DNA radioactivity, which is an index of DNA repair stimulation, and unscheduled DNA synthesis as measured by autoradiography were determined. The agents used fell into four groups: inhibitors of DNA replication, with evident absolute and relative increases in radioactivity, inhibitors of replication with relative but not absolute increases in radioactivity, compounds not altering any observed parameter, and inhibitors of replication giving negative absolute and relative increases in DNA radioactivity. The procedure described is an improved approach to screening chemicals able to induce DNA repair in mammalian cell cultures, which avoids time-consuming autoradiographic analysis when the scintillometric parameters are definitely positive or negative. (45 Refs)

28. POLY(ADENOSINEDIPHOSPHORIBOSE) POLYMERASE INHIBITORS STIMULATE UNSCHEDULED DEOXYRIBONUCLEIC ACID SYNTHESIS IN NORMAL HUMAN LYMPHOCYTES.

Sims JL, Sikorski GW, Catino DM, Berger SJ, Berger NA
(c/o N. A. Berger) Hematology/Oncology Div., Dept. Medicine, Washington Univ. Sch. Medicine, St. Louis, MO, 63110
Biochemistry; 21(8):1813-1821 1982

Thirty-three analogues of the nicotinamide and adenine portions of NAD+ were evaluated for (1) their effectiveness as inhibitors of poly(adenosinediphosphoribose) polymerase in nucleotide permeable lymphocytes, (2) their ability to interfere with NAD+ lowering in DNA-damaged cells, (3) their ability to inhibit accumulation of poly(adenosinediphosphoribose) in DNA-damaged cells, and (4) their ability to stimulate unscheduled DNA synthesis in DNA-damaged cells. Poly(adenosinediphosphoribose) polymerase was effectively inhibited by six-membered aromatic ring compounds containing a carboxamide group such as nicotinamide, picolinamide, 5-methylnicotinamide, benzamide, 3-aminobenzamide, and 5-methoxybenzamide. Compounds containing a carboxylic acid group rather than a carboxamide group did not inhibit poly(adenosinediphosphoribose) polymerase. Purine analogues such as theobromine and theophylline were also effective inhibitors of poly(adenosinediphosphoribose) polymerase. The ability of these compounds to inhibit poly(adenosinediphosphoribose) polymerase in nucleotide permeable cells correlated with their ability to retard NAD+ lowering in vivo in cells treated with N-methyl-N'-nitro-N-nitrosoguanidine. However, the most effective polymerase inhibitors caused the greatest retardation in the rate of NAD+ lowering. The compounds that were the most effective polymerase inhibitors in vitro were also the most potent in retarding NAD+ lowering in vivo and the most potent in delaying poly(adenosinediphosphoribose) accumulation in vivo. These compounds were also found to be the most effective stimulators of unscheduled DNA synthesis. For example, unscheduled DNA synthesis in UV-irradiated cells was stimulated two-fold by treatment with 2 mM benzamide, nicotinamide, or 3-aminobenzamide. (24 Refs)

29. ADAPTATION TO ALKYLATION RESISTANCE INVOLVES THE INDUCTION OF A DNA GLYCOSYLASE.

Evensen G, Seeberg E
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Nature; 296(5859):773-775 1982

The possibility of two pathways for repair of 3-methyladenine residues in Escherichia coli DNA was examined. The first pathway appears to be constitutively expressed and controlled by the gene tag, the second inducible and controlled by ada. Adaptive treatment with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), used to induce 6-methylguanine DNA methyltransferase, also induced high levels of 3-methyladenine DNA glycosylase activity in the tag mutant (BK2114). This induction was not seen in the tag ada double mutant (BK2106). BK2114 additionally regained its ability to reactivate alkylated phages. These results indicate the induction of two separate enzymes for repair of alkylated DNA: 6-methylguanine DNA methyltransferase and 3-methyladenine DNA glycosylase. Both of these enzymes appear under ada control. Since the ada mutation prevents induction of these two separate repair enzymes, the ada gene may be a control gene for the adaptive response. Structural genes for one or both enzymes may be situated elsewhere on the chromosome. Another mutant, alk-1, was found deficient in the induction of TagII but proficient in induction of the methyltransferase. 6-Methylguanine DNA methyltransferase is thought responsible for mutagenic adaptation, while ada+-dependent inducible glycosylase may be responsible for killing adaptation, because DNA polymerase I is required to complete repair of the apurinic site formed by glycosylase action. (14 Refs)

30. GENETIC AND BIOCHEMICAL CONSEQUENCES OF THYMIDYLATE STRESS.

Barclay BJ, Kunz BA, Little JG, Haynes RH
Dept. Biological Sciences, Brock Univ., St. Catherines, Ontario L2S 3A1, Canada
Can J Biochem; 60(3):172-194 1982

We have examined the genetic and biochemical consequences of thymidylate stress in haploid and diploid strains of the simple eukaryote Saccharomyces cerevisiae (Bakers' yeast). Previously we reported that inhibition of dTMP biosynthesis causes 'thymineless death' and is highly recombinagenic, but apparently not mutagenic, at the nuclear level; however, it is mutagenic for mitochondria. Concurrent provision of dTMP abolishes these effects. Conversely, excess dTMP is highly mutagenic for nuclear genes. It is likely that DNA strand breaks are responsible for the recombinagenic effects of thymidylate deprivation; such breaks could be produced by reiterative uracil incorporation and excision in DNA repair patches. In our experiments, thymidylate stress was produced both by starving dTMP auxotrophs for the required nucleotide and also by blocking de novo synthesis of thymidylate by various antimetabolites. We found that the antifolate methotrexate is a potent inducer of mitotic recombination (both gene conversion and mitotic crossing-over). This suggests that the gene amplification associated with methotrexate resistance in mammalian cells could arise, in part, by unequal sister-chromatid exchange induced by thymidylate stress. In addition, several sulfa drugs, which impede de novo folate biosynthesis, also have considerable recombinagenic activity. (Author abstract) (138 Refs)

31. POSTREPLICATION REPAIR IN NEUROSPORA CRASSA.

Calza RE, Schroeder AL
Program in Genetics and Cell Biology, Washington State Univ., Pullman, WA, 99164
MGG; 185(1):111-119 1982

Changes in the mol wt of nascent DNA made after ultraviolet (UV) irradiation have been studied in the excision-defective Neurospora mutant uvs-2 using isotopic pulse labeling, alkaline gradient centrifugation and alkaline filter elution. Both the size of nascent DNA and the rate of incorporation of label into DNA was reduced by UV light in a dose dependent manner. However, this DNA repair mutant did recover the ability to synthesize control-like high mol wt DNA 3 hr after UV treatment, although the rate of DNA synthesis remained depressed after the temporary block to elongation (or ligation) had been overcome. Photoreactivation partially eliminated the depression of DNA synthesis rate and UV light killing of cells, providing strong evidence that the effects on DNA synthesis and killing were caused by pyrimidine cyclobutane dimers. The caffeine inhibition repair studies performed were difficult to quantitate but did suggest either partial inhibition of a single repair pathway or alternate postreplication DNA repair pathways in Neurospora. No enhancement in killing was detected after UV irradiation when cells were grown on caffeine containing plates. (Author abstract) (57 Refs)

32. CLONED TRUNCATED RECA GENES IN E. COLI. I. EFFECT ON RADIOSENSITIVITY AND RECA+ DEPENDENT PROCESSES.

Sedgwick SG, Yarranton GT
Genetics Div., Natl. Inst. Medical Res., London NW7 1AA, England
MGG; 185(1):93-98 1982

Plasmids pMH1 and pDR1461, possessing the control region and 22% or 73% of the Escherichia coli recA gene, conferred UV sensitivity to wild-type uvrA, and umuC bacteria. Sensitization was less in recA441 (tif-1) mutants and absent in lexA cells. Radiosensitization correlated with inhibition of recombinational repair, even through induced recA protein synthesis and recombination in Hfr matings were normal. Plasmids pMH1 and pDR1461 also prevented induction of some, but not all, SOS functions. Mutagenic reversion to tryptophan prototrophy and induced reactivation of UV-irradiated phage lambda were eliminated, and the efficiency of lambda lysogenic induction reduced. However, nalidixic acid induced filamentous growth, mitomycin-C induced uvrA gene expression and post UV-irradiation DNA degradation control were little changed. Explanations of these effects are discussed which involve the presence of either truncated recA protein or multiple copies of the recA gene control sequence. (Author abstract) (41 Refs)

33. CLONED TRUNCATED RECA GENES IN E. COLI. II. EFFECTS OF TRUNCATED GENE PRODUCTS ON IN VIVO RECA+ PROTEIN ACTIVITY.

Yarranton GT, Sedgwick SG
Genetics Div., Natl. Inst. Medical Res., London NW7 1AA, England
MGG; 185(1):99-104 1982

The ability of plasmids carrying truncated recA genes to sensitize recA+ cells to UV-irradiation was dependent upon the size of the cloned recA gene fragment. Radiosensitization correlated with the inhibition of recombinational repair, and the in vivo reduction of recA protein recombinase activity, as measured by lambdabio11 plating efficiency. W-reactivation was also abolished by the radiosensitizing plasmids, while DNA degradation control, nalidixic acid induced filamentation and lambda induction were unaffected. UV-induced mutagenesis in excision proficient Escherichia coli was unaffected, while excision deficient strains were hypermutable. It is suggested that these effects of plasmids bearing 22% or more of the recA gene are the result of the interaction of full-sized and truncated protein subunits to generate multimers unable to catalyze recombination. (Author abstract) (14 Refs)

34. A BACTERIOPHAGE T4 MUTANT DEFECTIVE IN BOTH DNA REPLICATION AND REPLICATION REPAIR.

Cupido M, Schreij-Visser O, Van der Ree P
Dept. Radiation Genetics and Chemical Mutagenesis, Wassenaarseweg 72, Leiden, The Netherlands
Mutat Res; 93(2):285-295 1982

The bacteriophage T4 mutant uvs57 is characterized. The mutation causes arrest of DNA synthesis at 25 and 30 C. Mapping, crossing, multiplication, recombination, UV-sensitivity, fidelity and kinetics of DNA replication, and release of progeny DNA from the host-cell membrane are described. Repair of UV-induced lesions by T4 is discussed. (36 Refs)

35. MECHANISM OF TOXICITY OF PLATINUM (11) COMPOUNDS IN REPAIR-DEFICIENT STRAINS OF ESCHERICHIA COLI.

Alazard R, Germanier M, Johnson NP
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Mutat Res; 93(2):327-337 1982

Three platinum compounds, cis-platinum diamino dichloride (cis-PDD), trans-platinum diamino dichloride (trans-PDD), and [Pt(dien)Cl] Cl (dien = H2N-CH2-CH2-NH-CH2-CH2-NH2), were studied in terms of survival experiments with 10 strains of Escherichia coli, platinum binding to DNA immediately after treatment, inhibition of DNA synthesis, and repair synthesis and toxicity of these compounds in wild-type and repair-deficient E coli strains. The three Pt compounds bind to E coli DNA in vivo. The cis- and trans-PDD exert their toxic effect by inhibition of DNA synthesis. Wild-type bacteria are able to survive by partially repairing these DNA lesions, but mutant strains cannot accomplish this repair. The lower toxicities of trans-PDD and [Pt(dien)Cl] Cl are not due to a low level of DNA binding or to a greater repair of the damaged DNA, suggesting that E coli replication is unimpaired by some Pt(II)-chloramine-DNA adducts. The relative toxicities of the three types of DNA damage appear to depend on the structure of Pt-DNA adducts as well as on the number of lesions. (21 Refs)

36. RESIDENT ENHANCED REPAIR: NOVEL REPAIR PROCESS ACTION ON PLASMID DNA TRANSFORMED INTO ESCHERICHIA COLI K-12.

Strike P, Roberts J
Dept. Genetics, Univ. Liverpool, Liverpool L69 3BX, England
J Bacteriol; 150(1):385-388 1982

The survival of UV-irradiated DNA of plasmid NTP16 was monitored after its transformation into recipient cells containing an essentially homologous undamaged plasmid, pLV9. The presence of pLV9 resulted in a substantial increase in the fraction of damaged NTP16 molecules which survived in the recipient cells. This enhanced survival requires the host uvrA+ and uvrB+ gene products, but not the host recA+ gene product. The requirement for both homologous DNA and the uvrA+ and uvrB+ gene products suggests that a novel repair process may act on plasmid DNA. Possible mechanisms for this process are considered. (Author abstract) (14 Refs)

37. PHOTOPRODUCT FORMATION AND REPAIR CAPACITY IN A MUTANT OF BACILLUS CEREUS 569 PRODUCING UV-SENSITIVE SPORES.

Weinberger S, Evenchik Z, Hertman I
Israel Inst. Biological Res., Ness Ziona, Israel
Mutat Res; 94(1):1-12 1982

The isolation of N-methyl-N'-nitro-N-nitroso-guanidine-induced mutants of Bacillus cereus 569 sensitive to UV in both vegetative and sporal stages is reported. In this mutant (2422) a single mutation in a gene coding for a step in excision repair apparently leads to the observed sensitivity of both cells and spores. Dipicolinic acid content of spores of strain 2422 was similar to that of wild type, indicating that the mutation induced was not involved in the synthesis of this product. For a given UV dose, both the mutant 2422 and the wild type formed the same types and the same amount of photoproducts. The dominant photoproduct for vegetative cells was cyclobutane-thymine dimers, and for spores 5-thyminyl-5,6-dihydrothymine (TDHT). Under conditions permitting germination but no

outgrowth, only about 50% of TDHT lesions were removed; no significant difference was observed in rates of removal in the wild type vs the mutant strain. Under growing conditions only the wild-type 569 improved in rate of removal of TDHT. These findings suggest more than one pathway for TDHT removal. Under growing conditions plus energy source, the wild type 569 excised approx 75% of initial thymine dimers, compared to only 26% in the mutant. Reversion analysis indicated that UV resistance was regained by single gene mutation. This gene is apparently involved in the removal of bases or nucleotides, and plays a major role in the repair of UV-induced damage in both spores and vegetative cells. (29 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

38. SPECTROSCOPIC ANALYSIS OF DRUG-NUCLEIC ACID INTERACTIONS.

Dougherty G, Pigram WJ
Sch. Physics, Univ. Sains Malaysia, Penang, Malaysia
CRC Crit Rev Biochem; 12(2):103-132 1982

Treatment and analysis of drug binding to nucleic acids are reviewed to provide a perspective on the problems associated with the analysis and subsequent interpretation of drug binding data. Spectral series of drug-nucleic acids complexes in solution are recorded at different binding ratios. Constant total drug concentration, maintained either experimentally or by normalization of the spectra, gives best results. Different interpretations of the binding process predict various relationships between the binding parameters and the proposed program produces graphs appropriate to these relationships. Interpretation of data for simple single-species binding and for more than one independent bound species, cooperativity and anticooperativity binding, and the excluded site model are discussed. A computer program was tested using data published for ethidium bromide and actinomycin C3 (ACM). The ACM data deviated markedly from the theoretical fitted curve implying that additional complications are operating in ACM binding. Useful information can be obtained from careful interpretation of Scatchard plot of the binding parameters. Much of the nonlinearity in such a plot can be attributed to a binding overlap effect. The physical nature of the exclusion will not be revealed directly, since it will probably be a combination of actual size of the drug and distortion of the local DNA conformation. The presence of different binding sites may be of great importance in the recognition process involved in protein-nucleic acids interactions and in DNA replication and transcription, as well as in chemotherapeutic consideration. (71 Refs)

39. NONINTERCALATIVE DNA-BINDING ANTITUMOUR COMPOUNDS.

Baguley BC
Cancer Res. Lab., Univ. Auckland Medical Sch., Private Bag, Auckland, New Zealand
Mol Cell Biochem; 43(3):167-181 1982

A family of compounds which appear to bind reversibly to double stranded DNA without intercalation between DNA base pairs has been defined. Methods are described by which this non-intercalative binding can be characterized using ultraviolet spectrometry, fluorimetry with ethidium as a probe, viscometry and other hydrodynamic techniques, circular dichroism and nuclear magnetic resonance spectrometry. antibiotics which fall into this family include the antibiotics distamycin A, netropsin, mithramycin, chromomycin and olivomycin. Synthetic antitumor agents include diarylamidines such as berenil, phthalanilides, aromatic bisguanylhydrazones and bisquaternary ammonium heterocycles. A survey has been made of the general requirements of this family of compounds for DNA binding and biological activity. Binding of drugs to the minor groove of the DNA double helix appears to be the most likely mechanism for the antitumor action of these compounds (Author abstract) (91 Refs)

40. ADRIAMYCIN-INDUCED COMPACTION OF ISOLATED CHROMATIN.

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Biochem Pharmacol; 31(6):1057-1061 1982

Adriamycin and certain other agents such as daunomycin, rubidazone and 1,4-dihydroxyl-5,8 bis [[2-[(2-hydroxyethyl)amino]ethyl]amino]9,10 anthracenedione (DHAQ) were capable of inducing a considerable increase in the sedimentation rate of isolated chromatin. In contrast, N-trifluoroacetyl-adriamycin-14-valerate (AD-32) was without effect in this system. Electron microscope studies of chromatin incubated with adriamycin, rubidazone and DHAQ showed that all of these drugs were capable of inducing the formation of highly condensed DNA structures. When chromatin was treated with 0.4 M NaCl, its ability to serve as a substrate for adriamycin-induced compaction was abolished. However, the salt-treated chromatin could still be condensed by DHAQ and rubidazone. The results of this study, therefore, show that a variety of closely related antitumor agents are capable of condensing isolated chromatin. However, certain of these compounds had distinct substrate requirements for the compaction reaction. (Author abstract) (20 Refs)

41. THE FREQUENCY OF INTERSTRAND CROSS-LINKS IN DNA FOLLOWING REACTION OF CIS-DIAMMINEDICHLOROPLATINUM(II) WITH CELLS IN CULTURE OR DNA IN VITRO: STABILITY OF DNA CROSS-LINKS AND THEIR REPAIR.

Roberts JJ, Friedlos F
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Chem Biol Interact; 39(2):181-189 1982

DNA that had been reacted in vitro with cis-diamminedichloroplatinum(II) (cisplatin) had the same frequency of interstrand cross-linking (namely, approx 1 cross-link per 150 platinum reaction) as that found in the DNA isolated from cells in culture immediately after their treatment with cisplatin. Incubation of both the in vitro and in vivo platinum-reacted DNA in sodium citrate buffer for 24 hr at 37 C resulted in up to a five-fold increase in cross-link frequency. (Author abstract) (11 Refs)

42. INFLUENCE OF TEMPERATURE ON PLATINUM BINDING OF DNA, CELL KILLING, AND MUTATION INDUCTION IN ESCHERICHIA COLI K-12 CELLS TREATED WITH CIS-DIAMMINEDICHLOROPLATINUM(II).

Brouwer J, Fichtinger-Schepman AM, Van de Putte P, Reedijk J
Lab. Molecular Genetics, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands
Cancer Res; 42(6):2416-2419 1982

Cell killing and specificity of mutation induction by treatment of Escherichia coli K-12 cells with cis-Pt(NH3)2Cl2 at various temperatures have been studied. Survival experiments show that the cell killing by cis-Pt(NH3)2Cl2 is enhanced with increasing temperature. This effect is explained by an increase in the amount of platinum bound to the DNA. The binding is the same in repair-proficient and in repair-deficient cells. However, the mutation induction in the lacI gene is much more strongly enhanced than could be expected from the increased platinum binding. This phenomenon is attributed to a different distribution of the induced lesions by cis-Pt(NH3)2Cl2 at various temperatures and not to an aberrant excision repair. Analysis of the induced lacI mutants revealed an increase in the percentage of nonsense mutants at higher temperature. Among the nonsense mutations, base-pair substitutions at GAG and particularly at GCG sequences are enhanced by the increasing temperature. The results are in agreement with our hypothesis that local denaturation of DNA, known to be promoted at higher temperature, is necessary for the formation of intrastrand cross-links at two guanine bases separated by a third base. (Author abstract) (10 Refs)

43. SPECTROSCOPIC CHARACTERIZATIONS AND COMPARISONS OF THE STRUCTURES OF THE COVALENT ADDUCTS DERIVED FROM THE REACTIONS OF 7,8-DIHYDROXY-7,8,9,10-TETRAHYDROBENZO[A]PYRENE-9,10 OXIDE, AND THE 9,10-EPOXIDES OF 7,8,9,10-TETRAHYDROBENZO[A]PYRENE AND 9,10,11,12-TETRAHYDROBENZO[E]PYRENE WITH DNA.

Geacintov NE, Gagliano AG, Ibanez V, Harvey RG
Chemistry Dept., New York Univ., New York, NY, 10003
Carcinogenesis; 3(3):247-253 1982

The conformation of covalent adducts derived from the reactions of racemic 7beta,8alpha-dihydroxy-9alpha,10-alpha-epoxy-7,8,9,10 tetrahydrobenzo[a] pyrene (BaPDE), 9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BaPE), and 9,10-epoxy-9,10,11,12-tetrahydrobenzo[e]pyrene (BePE) with calf thymus DNA in aqueous buffer solution (25 C, pH 7.0) were investigated and compared by means of absorption, fluorescence and electric linear dichroism techniques. Two types of conformations are recognized. Site I is characterized by a red shift (approx 10 nm) in the absorption max of the pyrene nucleus, a significantly reduced fluorescence yield, and a negative electric linear dichroism signal (DeltaA); this site is presumed to involve a near-parallel (within 25 C) orientation of the planar pyrene residue with the planes of the DNA bases, and a relatively strong interaction between the pi electrons of the nucleic acid bases and the pyrene residue. In Site II, there is only a small red-shift in the absorption max (approx 2 nm), a nonzero fluorescence yield, and a postive DeltaA throughout the absorption region of the pyrene residue; in this conformation the pyrene residue is presumed to lie on the outside of the DNA molecule, possibly in one of the grooves. The BaPDE-DNA complex displays predominantly a Site II-type conformation while the BaPE- and BePE-DNA complexes display both Site I and Site II adducts, with Site I, conformations predominating. The lack of hydroxyl groups in BaPE and BePE lead to a loss in stereospecificity in covalent adduct formation. The 7 and 8 hydroxyl groups in BaPDE appear to reduce the probability of formation of Site I-type of covalent adducts, and appear to be, at least in part, responsible for the enantiomeric stereospecificity in the covalent reaction between BaPDE and DNA. (Author abstract) (25 Refs)

44. THE STRUCTURE OF A PSEUDO INTERCALATED COMPLEX BETWEEN ACTINOMYCIN AND THE DNA BINDING SEQUENCE D(GPC).

Takusagawa F, Dabrow M, Neidle S, Berman HM
Inst. Cancer Res., Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA, 19111
Nature; 296(5856):466-469 1982

Crystallographic analysis of the 2:1 complex between deoxyguanylyl-3', 5'-deoxycytidine [d(GpC)] and actinomycin D (AMD) indicated that the complex forms an unusual pseudo-intercalated structure. The principal and surprising feature of the crystal structure is that it does not contain the intercalated duplex structure previously proposed. Instead, the d(GpC) molecule is in the opened-out conformation with the phosphodiester torsion angles in the g+g+ range rather than the g-g- range found in self-complementary duplexes. Explanations for the unusual crystal structure of the d(GpC)-AMD complex are discussed. (29 Refs)

45. ANALYSIS OF N-ACETOXY-N-2-ACETYLAMINOFLUORENE-INDUCED FRAME-SHIFTS IN PBR322.

Yoon K, Shelegedin VN, Kallenbach NR
Center Oral Health Res., Sch. Dental Medicine, Univ. Pennsylvania, Philadelphia, PA, 19104
Mutat Res; 93(2):273-283 1982

A simple system is described which can be used to study directly the sequence change and cellular functions involved in frame-shift mutagenesis by covalently reactive mutagen. Positive and negative alterations in the number of base pairs of tetracycline resistant gene of pBR322, were created by cutting with Sal restriction enzyme followed by either removal with nuclease Sl or extension with reverse transcriptase, and reclosing by a 'blunt-ended' joining reaction catalyzed by T4 DNA ligase. Mutagenesis in vitro by exposure to N-acetoxy-N-2-acetylaminofluorene (AAAF) was used to cause reversion of frame-shifted plasmid molecules. Transformation of AAAF-reacted frame-shift plasmid DNA into Escherichia coli strains AB1157, AB1886, and AB2463 was performed, and a number of revertants (ApRTcR) not represented in unreacted controls were generated. Differential inactivation of the AP gene was observed among the E coli strains. The system used here is suitable for any chemicals that bind covalently to DNA. (25 Refs)

46. MECHANISM FOR THE LOSS OF PREFERENTIAL BENZO[A]PYRENE BINDING TO THE LINKER DNA OF CHROMATIN.

Jack PL, Brookes P
Chemical Carcinogenesis Div., Inst. Cancer Res., Pollards Wood Res. Station, Nightingales Lane, Chalfont St. Giles, Bucks HP8 4SP, England
Carcinogenesis; 3(3):341-344 1982

We have examined the fate of the asymmetric chromosomal distribution of DNA adducts generated by the chemical carcinogen r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10 tetrahydrobenzo[a]pyrene (BPDE). Treatment of mouse embryo cells with BPDE results in 3.5x more binding to the linker DNA regions between nucleosome cores than to the nucleosome core DNA itself, but 24 hr post-treatment incubation of these cells leads to a loss of this non-random binding. A similar result was obtained when post-treatment incubation was carried out in the presence of hydroxyurea indicating that factors other than DNA replication are responsible for this change in adduct distribution. However in the case of excision repair deficient xeroderma pigmentosum (XP12/BE) cells the non-random adduct distribution was stable over a period of 48 hr, whereas with excision repair proficient XP variant (XP4/BE) cells, loss of preferential binding did occur. These results indicate that the loss of non-random nucleosomal DNA modification with time can be accounted for by the preferential removal of adducts from micrococcal nuclease sensitive linker DNA and further, demonstrates that in certain cells at least, the relative position of nucleosome core structures on DNA remains unchanged over a period of at least 48 hr. (Author abstract) (18 Refs)

47. NONENZYMATIC METHYLATION OF DNA BY S-ADENOSYLMETHIONINE IN VITRO.

Barrows LR, Magee PN
Fels Res. Inst., Philadelphia, PA, 19140
Carcinogenesis; 3(3):349-351 1982

S-Adenosylmethionine was found to methylate DNA nonenzymatically to produce the same putative promutagenic and procarcinogenic lesions formed by carcinogenic chemical methylating agents. The formation of 7-methylguanine was confirmed by uv spectrophotometry, the formation of O6-methylguanine and 3-methyladenine was suggested by the cochromatography of radioactivity with standard bases. It is possible that this reaction may explain the presence of constitutive cellular enzymes specifically for the repair of methylated DNA, and may indicate the mechanism whereby methylated guanine is formed in the liver DNA of rats with chemically induced liver damage. (Author abstract) (16 Refs)

48. MOVABLE GENETIC ELEMENTS.

Watson JD
Cold Spring Harbor Lab., Box 100, Cold Spring Harbor, NY, 11724
Cold Spring Harbor Symp Quant Biol 45(part 1): 445 pp., 1981.

Inversion elements in bacteria, general properties of transposable elements in bacteria (TEB), structure and function of TEB, factors affecting transposition, mechanism of transposition, mechanism of Mu transposition, and biochemistry of recombination are reviewed. Analysis of crosses between genetically marked corn plants has led to the postulate that activity of key genes is under the control of genetic elements having the capacity to move from one chromosomal site to another. When a control element moves away from a gene, the activity of that gene suddenly reappears. Certain highly pleiotropic mutations of Escherichia coli are the result of the insertion of discrete DNA segments that can jump from one chromosomal site to another. Insertion sequences (IS) may exist not only in bacteria but also in all organisms. Closely spaced pairs of IS can move as units, carrying along the genes lying between them. Such transposons bare many similarities with the phage Mu. Some experiments have indicated that the structure of integrated retroviruses is remarkably similar to that of transposons. (165l Refs)

49. CHROMOSOMAL LOCATION OF A HUMAN ALPHA INTERFERON GENE FAMILY.

Slate DL, D'Eustachio P, Pravtcheva D, Cunningham AC, Nagata S, Weissmann C, Ruddle FH
Cancer Metastasis Research Group, Pfizer Central Research, Groton, CT, 06340
J Exp Med; 155(4):1019-1024 1982

A series of human/rodent somatic cell hybrids were scored for the presence of DNA sequences hybridizing to an alpha-1 interferon DNA probe. Hybrids were generated by fusing Chinese hamster E36 cells with human BD melanoma cells, temperature-sensitive Chinese hamster ovary cells and human WBC, mouse A9 cells with GM17 human fibroblasts, or mouse A9 cells with GM589 human fibroblasts. Hybrids were karyotyped and tested for the presence of human marker isozymes. In human/Chinese hamster hybrid cells, sequences hybridizing to the human alpha1 interferon probe were clearly observed in cellular DNA when chromosome 9 was retained. In hybrids lacking chromosome 9, no human-specific bands were observed. Segregation of chromosome 9 below a frequency of 20-30% resulted in a failure to detect alpha1 interferon related sequences. With appropriate cell hybrids, it should be possible to map rodent alpha interferon-related DNA sequences. (25 Refs)

50. ISOLATION AND PRELIMINARY CHARACTERIZATION OF A HUMAN TRANSFORMING GENE FROM T24 BLADDER CARCINOMA CELLS.

Goldfarb M, Shimizu K, Perucho M, Wigler M
Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724
Nature; 296(5856):404-409 1982

DNA from T24, a cell line derived from a human bladder carcinoma, can induce the morphological transformation of NIH 3T3 cells. Using techniques of gene rescue to clone the gene responsible for this transformation, we have found that it is human in origin, less than 5 kilobase pairs in size and is homologous to a 1,100-base polyadenylated RNA species found in T24 and HeLa cells. Blot analysis indicates extensive restriction endonuclease polymorphism near this gene in human DNAs. (Author abstract) (26 Refs)

51. CELLULAR SEQUENCES RELATED TO THREE NEW ONC GENES OF AVIAN SARCOMA VIRUS (FPS, YES, AND ROS) AND THEIR EXPRESSION IN NORMAL AND TRANSFORMED CELLS.

Shibuya M, Hanafusa H, Balduzzi PC
Rockefeller Univ., New York, NY, 10021
J Virol; 42(1):143-152 1982

Two onc genes of avian sarcoma viruses unrelated to the src gene have recently been identified: fps of Fujinami sarcoma virus/PRCII/UR1 and yes of Y73/Esh sarcoma virus. In the first part of this study we demonstrated that UR2, the most recently isolated avian sarcoma virus, contains in its genome a unique sequence, ros, nonhomologous to src, fps, and yes sequences or to transforming genes of avian acute leukemia vir-

uses. Using cDNAs specific to the inserts of avian sarcoma virus genomes, we examined the existence and the transcription of cellular nucleotide sequences related to the three new onc genes of avian sarcoma virus (fps, yes and ros) in various cells. The progenitor cellular sequences for these onc genes (c-onc) were present in uninfected chicken DNA in one or few copies per haploid genome. These c-onc sequences were detectable in cellular DNA of a wide variety of vertebrates, and the homology between viral and cellular onc was inversely related to the phylogenetic distance of animal species. The pattern of expression of these c-onc genes in different tissues of chickens was found to be unique to each gene. The expression of c-fps and c-ros genes was generally repressed in many tissues, but c-fps was expressed at higher levels in bone marrow (2.5 copies per cell) and lung (1.1 copies per cell), whereas c-ros was mainly transcribed in kidney (2.5 copies per cell). On the other hand, c-yes transcripts were easily detectable in all tissues analyzed and were found at high levels in kidney (26 copies per cell). These c-onc expressions were unaffected by infection with avian sarcoma viruses that contained other onc genes. In a few cultures of chicken and quail transformed cells derived from tumors induced by chemical carcinogens, we found that the levels of transcription of the four c-onc genes remained unaltered, compared with that in normal tissues. (Author abstract) (60 Refs)

52. EXPRESSION OF CELLULAR HOMOLOGUES OF RETROVIRAL ONC GENES IN HUMAN HEMATOPOIETIC CELLS.

Westin EH, Wong-Staal F, Gelmann EP, Favera RD, Papas TS, Lautenberger JA, Eva A, Reddy EP, Tronick SR, Aaronson SA, Gallo RC

Lab. Tumor Cell Biology, NCI, NIH, Bethesda, MD, 20205

Proc Natl Acad Sci USA; 79(8,part1):2490-2494 1982

Total cellular poly(A)-enriched RNA from a variety of fresh human leukemic blood cells and hematopoietic cell lines was analyzed for homology with molecularly cloned DNA probes containing the ONC sequence of Abelson murine leukemia virus (Ab-MuLV), Harvey murine sarcoma virus (Ha-MuSV), simian sarcoma virus (SSV), and avian myelocytomatosis virus strain MC29. Results with the fresh blood cells paralleled those obtained with the cell lines. With Ab-MuLV and Ha-MuSV, multiple RNA bands were visualized in all cell types examined without significant variation in the relative intensities of the bands. When SSV was used as the probe, expression of related onc sequences was absent in all of the hematopoietic cell types examined except for one neoplastic T-cell line (HUT 102), which produces the human T-cell leukemia (lymphoma) retrovirus HTLV. In this cell line, a single band (4.2 kilobases) was observed. With MC29 as the probe, a single band of 2.7 kilobases was visualized in all cell types examined with only a one- to two-fold variation in intensity of hybridization. An exception was the promyelocytic cell line, HL60, which expressed approximately 10-fold more MC29-related onc sequences. With induction of differentiation of HL60 with either dimethyl sulfoxide or retinoic acid, a marked diminution in the amount of the MC29-related, but not the Ab-MuLV-related, onc message was observed. (Author abstract) (62 Refs)

53. CLONED CDNA SEQUENCES OF THE HYPOXANTHINE/GUANINE PHOSPHORIBOSYLTRANSFERASE GENE FROM A MOUSE NEUROBLASTOMA CELL LINE FOUND TO HAVE AMPLIFIED GENOMIC SEQUENCES.

Brennand J, Chinault AC, Konecki DS, Melton DW, Caskey CT
Howard Hughes Medical Inst. Lab., Baylor Coll. Medicine, Houston, TX, 77030

Proc Natl Acad Sci USA; 79(6,part1):1950-1954 1982

Cloned cDNA sequences of the murine hypoxanthine/guanine phosphoribosyltransferase (HPRT; EC 2.4.2.8) gene have been isolated by using a mouse neuroblastoma cell line containing increased levels of a variant HPRT protein. We have used these sequences as probes to demonstrate that protein over-production in this cell line is a consequence of at least a 20-fold increase in HPRT mRNA levels resulting from approximately 50-fold amplification of HPRT genomic sequences. The largest cDNA insert so far characterized represents about 70% of the HPRT mRNA sequence. This cDNA is shown to possess regions of homology with mRNA and DNA from Chinese hamster, baboon, and human, thus facilitating detailed analysis of this locus in these four species. (Author abstract) (33 Refs)

54. STRUCTURE OF GENES FOR MEMBRANE AND SECRETED MURINE IGD HEAVY CHAINS.

Cheng HL, Blattner FR, Fitzmaurice L, Mushinski JF, Tucker PW
Dept. Microbiology, Univ. Texas Southwestern Medical Sch., Dallas, TX, 75235

Nature; 296(5856):410-415 1982

We have sequenced secreted and membrane terminal exons of the murine immunoglobulin delta chain. The putative transmembranal exon is strikingly similar to that of the mu chain and the internal cytoplasmic exon codes for exactly the same two amino acids, valine and lysine. A third potential exon was found between S and M exons that could code for yet another hydrophilic carboxyl terminus termed deltaX. (Author abstract) (46 Refs)

55. TRANSFORMATION WITH DNA FROM 5-AZACYTIDINE-REACTIVATED X CHROMOSOMES.

Venolia L, Gartler SM, Wassman ER, Yen P, Mohandas T, Shapiro LJ
(c/o Gartler) Dept. Genetics, Medicine, Center Inherited Diseases, Univ. Washington, Seattle, WA, 98195

Proc Natl Acad Sci USA; 79(7):2352-2354 1982

It has been shown that 5-azacytidine (5-Aza-Cyd) can reactivate genes on the inactive human X chromosome. It is assumed that the 5-Aza-Cyd acts by causing demethylation of the DNA at specific sites, but this cannot be demonstrated directly without a cloned probe. Instead, we have utilized the technique of DNA-mediated transformation to show that the 5-Aza-Cyd-induced reactivation occurs at the DNA level. DNAs from various mouse-human or hamster-human hybrid cell lines, deficient for mouse or hamster hypoxanthine phosphoribosyltransferase (HPRT; EC2.4.2.8), and varying in whether they contained either an active or inactive human X chromosome, were used in transformation of HPRT- cells. DNA from the active human X chromosome-containing cell lines yielded HPRT+ transformants, whereas DNA from the inactive X chromosome-containing cell lines did not. The inactive X chromosomal DNA was able to transform thymidine kinase-deficient mouse cells, indicating that the DNA solution was normal. These results confirm that inactivation of the X chromosome involves a DNA modification. Furthermore,

DNAs from three cell lines with a 5-Aza-Cyd-reactivated X chromosome also transform HPRT- cells, demonstrating that the 5-Aza-Cyd has altered the DNA structure and supporting the idea that methylation plays a role in X chromosome inactivation. (Author abstract) (22 Refs)

56. DNA TRANSFER OF FOCUS- AND TUMOR-FORMING ABILITY INTO NONTUMORIGENIC CHEF CELLS.

Smith BL, Anisowicz A, Chodosh LA, Sager R
(c/o Sager) Dept. Microbiology and Molecular Genetics, Harvard Medical Sch., Boston, MA, 02115
Proc Natl Acad Sci USA; 79(6,part1):1964-1968 1982

CHEF/18 fibroblastic cells derived from a Chinese hamster embryo are diploid and nontumorigenic and require multiple steps of chemical treatment and selection to produce tumorigenic derivatives. In this report, CHEF/18 cells and a mutant capable of growing in medium with a low concentration of serum, LS1-1, were recipients in DNA transfer experiments using the calcium phosphate coprecipitation method. Focus formation with donor DNAs from tumor-derived CHEF cells and from human bladder carcinoma cell line EJ gave a yield of 0.02-0.59 focus per ug of DNA per 10(6) recipients. IN one experiment in which CHEF/18 cells were transfected with EJ DNA, the presence of human DNA was detected in five of seven foci by using a cloned Alu sequence. Cells from one of these foci gave rise to tumors in nude mice, and the DNA produced secondary CHEF/18 transfectants. Because normal human cells as well as CHEF/18 cells require multiple stages to become tumorigenic, these findings suggest that EJ cells contain tumor-inducing DNA as the result of prior changes that occurred during the development of this carcinoma. (Author abstract) (39 Refs)

57. GENE TRANSFER INTO THE MOUSE GERM-LINE.

Costantini F, Lacy E
Dept. Zoology, Univ. Oxford, Oxford OX1 3PS, England
J Cell Physiol; Suppl1:219-226 1982

Introduction of a cloned rabbit beta-globin gene into mice by microinjection of fertilized eggs is described. The DNA used consisted of a 19 kilobase (kb) rabbit DNA fragment containing the adult beta-globin gene and a beta-like pseudogene, which is cloned in the bacteriophage lambda vector CH4A. Fifty-one mice were obtained from eggs that had been injected with a mixture of linear monomeric lambdaRbetaG2 DNA (80-90%) and concatemeric lambdaRbetaG2 DNA (10-20%). Nine of 24 screened mice contained copies of lambdaRbetaG2 in liver DNA. Of the nine mouse DNA samples, five showed a hybridization pattern characteristic of lambdaRbetaG2 DNA (lanes and l), while the other four showed no hybridization to the rabbit beta-globin probe. Four male and two female mice tested transmited the rabbit DNA fragments to a fraction of their offspring. The transmission of lambdaRbetaG2 sequences by one mouse to two out of six of its progeny was demonstrated. When the mouse DNA was digested with EcoRI (which cleaves the 19 kb and 10.7 kb lambda vector arms from the rabbit DNA insert) and probed with 32P-labeled lambdaRbetaG2, a 30 kb band was seen in every case instead of the 19 kb and 10.7 kb bands. Most of the mouse DNA showed a few fainter new EcoRI bands that hybridize with the lambdaRbetaG2 probe. These bands were of different mol wt in each of the original positive mice and were inherited by the progeny. Definite evidence for chromosomal integration was obtained for one parent mouse by in situ hybridization to metaphase chromosome spreads prepared from peripheral blood. (27 Refs)

OTHER RELATED STUDIES

58. CYTOMETRIC EVIDENCE OF CYTOGENETIC AND PROLIFERATIVE HETEROGENEITY OF HUMAN SOLID TUMORS.

Nervi C, Badaracco G, Maisto A, Mauro F, Tirindelli-Danesi D, Starace G
Istituto Medico e di Ricerca Scientifica, 00191 Rome, Italy
Cytometry; 2(5):303-308 1982

Eleven cases of non-small cell carcinoma of the lung (primary or metastasis of different histological type) and 3 cases of non-neoplastic lung disease were studied by flow cytometry. The modal DNA content expressed as DNA index is reported. Except in one case all neoplastic lesions were characterized by the concomitant presence of a diploid peak with a modal DNA content higher than diploid. Aneuploidy was scattered apparently at random, with DNA index in the range 1.20-3.50. The three non-neoplastic cases showed normal diploid DNA content. In 3/11 neoplastic cases multiple clones of different DNA index were identified in more than one sample from the same tumor. The relative proportion of cells in the diploid and aneuploid peaks varied between tumors and in samples from different areas of the same tumor. Due to overlapping of normal and neoplastic DNA distributions or to the presence of overlapping multiple DNA distributions, the evaluation of cell fractions in the cycle phases was performed in only 3/11 neoplastic cases. The main problem was in obtaining reliable estimations in specimens characterized by a DNA distribution of complex structure. Flow cytometry has been applied routinely to analyze mainly head and neck squamous cell carcinoma and breast carcinoma. (11 Refs)

59. DEOXYCOFORMYCIN-INDUCED RESPONSE IN CHRONIC LYMPHOCYTIC LEUKAEMIA: DEOXYADENOSINE TOXICITY IN NON-REPLICATING LYMPHOCYTES.

Kefford RF, Fox RM
Ludwig Inst. Cancer Res., Univ. Sydney, Blackburn Building, Sydney, N.S.W. 2006, Australia
Br J Haematol; 50(4):627-636 1982

The occurrence of severe immunodeficiency disease in children with inherited adenosine deaminase deficiency, and reports of remission induction in T-cell acute lymphoblastic leukemia with the adenosine deaminase inhibitor deoxycoformycin, prompted a study of the effects of deoxyadenosine on resting peripheral blood lymphocytes (PBL) and chronic lymphocytic leukemic (CLL) lymphocytes in short-term culture. In the presence of an inhibitor of adenosine deaminase, micromolar concentrations of deoxyadenosine caused evolution deoxyadenosine-5'-triphosphate (dATP) pools and in vitro lysis of non-dividing PBL and CLL lymphocytes. This death of non-replicating cells indicates a mechanism of deoxyadenosine toxicity independent of DNA replication and ribonucleotide reductase inhibition. Similar changes occurred in vivo in a patient with advanced CLL who responded to treatment with deoxycoformycin, 0.1 mg/kg, days 1-5, with a fall in the WBC from 102 x 10(9)/l to 6.8 x 10(9)/l over 21 days. Therapeutic blockade of deoxyadenosine catabolism deserves further investigation both in the treatment of lymphoproliferative disease and as a method lympholytic immunosuppression. (Author abstract) (20 Refs)

* U.S. GOVERNMENT PRINTING OFFICE : 1982—361-547/125

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CHARLES HEIDELBERGER University of Southern California	*Mutagenesis and Oncogenic Transformation of Mouse Embryo Cells*	#VK04 54 min.
FRED RAPP Milton S. Hershey Medical Center	*The Interaction of Herpesviruses and Host Cells Leading to Latency or Transformation*	#VK05 52 min.
	Panel Discussion	#VK06 31 min.

MECHANISMS OF METASTASIS

ISAIAH FIDLER Frederick Cancer Research Center	*The Heterogeneous Nature of Cancer Metastasis and Implications for Therapy*	#VB01 47 min.
GEORGE POSTE Smith, Kline and French Laboratories	*Phenotypic Analysis of Metastatic Cell Populations*	#VB02 42 min.
LANCE LIOTTA National Cancer Institute	*Tumor Cell Invasion of Basement Membranes and Extracellular Matrices*	#VB03 33 min.
EVERETT SUGARBAKER Miami Cancer Institute	*Some Clinical Characteristics of Metastasis in Man*	#VB04 41 min.

AUTOMATED CYTOLOGY

Part One: Principles and Techniques	#VB05 56 min.
Part Two: Biological Applications	#VB06 52 min.

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DNA

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MECHANISMS OF DNA REPLICATION

1. THE ROLE OF RNA PRIMER IN DISCONTINUOUS DNA REPLICATION IN ISOLATED NUCLEI FROM HELA CELLS.

Wang ML, Friedman DL
(c/o Friedman) Dept. Molecular Biology, Vanderbilt Univ., Nashville, TN, 37235
Biochim Biophys Acta; 697(1):41-52 1982

RNA-primed discontinuous DNA synthesis was studied in an in vitro system consisting of washed nuclei from synchronized S-phase HeLa cells. A new technique proved useful for the purification of short nascent fragments of DNA (Okazaki fragments). Mercurated dCTP was substituted for dCTP in the DNA synthesis reaction. Short nascent pieces (4-6 S) of mercurated DNA were found to bind preferentially to sulfhydryl-agarose, and could be eluted with mercaptoethanol. The isolated fragments were assayed for the presence of covalently linked RNA by the spleen exonuclease method described by Kurosawa et al. Following a 30 s incubation with [3H]TTP in the absence of added ribonucleotides, approx 20% of the nascent strands synthesized in washed nuclear preparations had

HE 20.3173/2:

RNA attached. These RNA primers either preexisted in the nuclei or were formed from endogenous ribonucleotides. The 5' ends of the primers appeared to be largely in a phosphorylated state. In the absence of added ribonucleotides, these RNA-DNA linkages disappeared within 2 min, whereas if ribonucleotides were added, the number of RNA primers increased to 40% and remained at this level for greater than 2 min. To obtain maximal levels of RNA primer, the addition of all three of the ribonucleotides, rCTP, rGTP and rUTP (0.1 mM), as well as high levels of rATP (5 mM) was required. Addition of ribonucleotides also markedly enhanced the amount of nascent DNA fragments synthesized. However, in the absence of added ribonucleotides, after RNA primers had disappeared, nascent DNA fragments were still initiated at a significant rate. These results suggest that RNA primers play an important role in the initiation of Okazaki fragments but that synthesis can also be initiated by alternative mechanisms. An important role for ATP in RNA primer synthesis is suggested. (Author abstract) (37 Refs)

2. EVIDENCE FOR THE RESUMPTION OF DNA REPLICATION PRIOR TO HISTONE SYNTHESIS IN HELA CELLS AFTER RELEASE FROM TREATMENT WITH HYDROXYUREA.

Shephard EA, Phillips I, Davis J, Stein JL, Stein GS
Dept. Biochemistry, Univ. Coll. London, Gower St., London WC1E 6BT, England
FEBS Lett; 140(2):189-192 1982

Histone and DNA synthesis were examined at various times after removal of hydroxyurea (HU) from HeLa cell cultures because of the potential value of HU for assessing the apparent functional coupling of DNA replication and histone synthesis. Greater than 90% inhibition of both DNA and histone synthesis occurred during HU treatment. When cells were treated for 30 min with 10 milliM of HU and then resuspended in fresh medium, DNA synthesis recovered to control levels within 10 min; however, histone synthesis did not return to control levels until 60 min after HU removal. When HeLa cells were treated with 10 milliM of HU for 2 hr and then resuspended in fresh medium, DNA synthesis rapidly (within 10 min) returned to control levels. Although inhibition of histone synthesis was completely reversible following 2 hr of HU treatment, the initial rate at which histone synthesis resumed was higher for cells treated with HU for 30 min than for cells treated for 2 hr. Histones were synthesized at control levels 60 min after release from the 2-hr drug block. (12 Refs)

EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

3. EFFECTS OF SERUM DEPRIVATION ON THE INITIATION OF DNA SYNTHESIS IN THE SECOND GENERATION IN RAT 3Y1 CELLS.

Okuda A, Kimura G
Inst. Cancer Res., Kyushu Univ. Sch. Medicine, Maidashi, Fukuoda 812, Japan
J Cell Physiol; 110(3):267-270 1982

Serum-dependent events continuing in the first generation may be a prerequisite for normal entry into S phase in the second generation in untransformed fibroblasts. It is reported that rat 3Y1 cells which have traversed S and G2 phases in the absence of serum, require additional time to enter S phase in the second generation. Serum deprivation did not induce any damage that hindered traverse through G1. (16 Refs)

4. DNA SYNTHESIS IN DIFFERENTIATING NEUROBLASTOMA CELLS FOLLOWING INDUCTION BY GLIOMA CELLS.

Allin EP, Wittendorp-Rechenmann E, Winsor B, Rechenmann RV
Laboratoire de Physiologie et de Biologie Cellulaire, Universite Louis Pasteur, Strasbourg, France
Cell Mol Biol; 28(1):37-41 1982

Transient co-culture of neuroblastoma cells with glioma cells has been used to induce differentiation in the neuroblastoma. The rate of DNA synthesis has been measured for 6 days following induction of differentiation. The initial rate of synthesis in differentiating cells is less than half that of the controls and the rate is maintained over the 6 days while that of the control cells falls to a low level from the 3rd day of culture. The distribution of label in induced and in non-induced cells has been followed by autoradiography. On the 6th day of culture 70% of the induced cells appear labeled while 40% of the non-induced cells show labeled nuclei. The induced cells are morphologically differentiated while the non-induced cells show few axon-like processes. (Author abstract) (17 Refs)

5. AN INHIBITOR OF DNA-SYNTHESIS, ISOLATED FROM RAT TUMOR-INDUCED ASCITES.

Wallevik K, Jensenius JC, Larsen JK, Kroll J, Lassota H
Finsen Lab., Finsen Inst., 49, Strand Blvd. 24/2, DK-2100 Copenhagen, Denmark
Virchows Arch [Cell Pathol]; 39(2):145-158 1982

An inhibitor of DNA-synthesis in rat Yoshida hepatoma cells in vitro was isolated from the corresponding ascites tumor by phosphocellulose chromatography. The inhibition is non-specific as the isolated fraction also inhibited normal rat thymus and spleen cells as well as other hepatomas and rat Walker carcinoma. Fractions isolated from Walker ascites and pooled normal rat serum have inhibitory properties similar to the inhibitor fractionated from Yoshida ascites by the same procedures. The inhibitory activity isolated from rat serum is significantly less than the one isolated from the Yoshida ascites. The cell lines examined showed gross variation in sensitivities to the inhibitor. This is, however, a characteristic of the cell rather than the inhibitor. The demonstrated variations in the responses among the cell lines to one inhibitor brings seriously into question the tissue-specificity of many growth inhibitors claimed to possess this property. The present findings emphasize the requirement for tests of tissue specificity of an inhibitor of cell growth: (1) The growth of at least three cell types from tissues different from the one in question must be shown to be unaffected by the inhibitor, and most important, (2) A fraction isolated from a different tissue by exactly the same procedure must leave the original cells uninhibited whether or not it inhibits the cells of the donor tissue. The inhibitor isolated from rat ascites and serum delays the initiation of the DNA-synthesis in resting cells that are stimulated to regrowth. It has an apparent mol wt of around 100,000 daltons, is relatively resistant to heat (100 C), but is destroyed by subtilisin. (Author abstract) (17 Refs)

6. SUPPRESSION OF HUMAN LYMPHOCYTE DNA AND PROTEIN SYNTHESIS IN VITRO BY ADENOSINE AND EIGHT MODIFIED ADENINE NUCLEOSIDES IN THE PRESENCE OR IN THE ABSENCE OF ADENOSINE DEAMINASE INHIBITORS, 2'-DEOXYCOFORMYCIN (DCF) AND ERYTHRO-9-(2-HYDROXY-3-NONYL) ADENINE (EHNA).

Ratech H, Kuritsky L, Thorbecke J, Hirschhorn R
Dept. Pathology, New York Univ. Sch. Medicine, New York, NY, 10016
Cell Immunol; 68(2):244-251 1982

Adenosine and eight modified adenine nucleosides in the presence or in the absence of adenosine deaminase (ADA) inhibitors, 2'-deoxycoformycin or erythro-9-(2-hydroxy-3-nonyl) adenine, were investigated with regard to their effects on phytohemagglutinin-stimulated human lymphocytes in vitro. Protein and DNA synthesis were inhibited depending upon the specific nucleoside, presence or absence of ADA inhibitor, and time of addition of nucleoside to cultures. A possible role for the modified adenine nucleosides in causing in vivo immunodeficiency is proposed. (Author abstract) (39 Refs)

7. EARLY PROLIFERATIVE CHANGES IN GASTROINTESTINAL NEOPLASIA.

Deschner EE
Lab., Digestive Tract Carcinogenesis, Memorial Sloan Cancer Center, 1275 York Ave., New York, NY, 10021
Am J Gastroenterol; 77(4):207-211 1982

The proliferative behavior of mucosal epithelium is reviewed in relation to the development of gastrointestinal cancer. An enlarged proliferative compartment has been recognized among individuals with a previous history of colon cancer, those with an isolated polyp, asymptomatic relatives of familial polyposis patients, those with ulcerative colitis, and even some members of the general population. Mucosa of patients with a previous history of colon cancer may show both extension of the proliferative compartment to the luminal surface (Stage I defect) and a shift in the major zone of DNA synthesis from the lower third of the gland to the middle and upper portion of the crypt (Stage II defect). Studies of 1,2-dimethylhydrazine-induced colon carcinogenesis in mice indicate that Stage II abnormalities are predictive of the development of cancer. Distribution of DNA synthesizing cells among patients belonging to several high risk groups is presented. Only 30% of the patients showed a completely normal proliferative pattern. Changes in proliferative patterns over time are described for two asymptomatic members of polyposis coli families. Histologically normal mucosa from patients with a previous history of neoplasia may show crypts with extremely elevated labeling indices. The importance of such crypts is discussed. Correlation of an abnormal proliferative pattern with the type of tumor that will emerge is described. The impact of a diet rich in animal and dairy fats is considered. (16 Refs)

8. IMPAIRMENT OF METABOLISM OF PURINE NUCLEOTIDES IN LYMPHOID AND HEMATOPOIETIC CELLS OF TUMOR-BEARING ANIMALS.

Vornovitskaia GI, Khramtsova SN, Nuzhnaia TV, Shapot VS
All-Union Cancer Res. Center, Moscow, USSR
Vestn Akad Med Nauk SSSR; (3):29-35 1982

Characteristic features of the metabolism of purine nucleotides were studied in the spleen and thymus lymphocytes and in peripheral blood and spleen RBC of rats with transplanted ascites Zajdela hepatoma and of mice with transplanted solid hepatoma 22. Marked decrease of adenosine deaminase (AD) activity in the thymus was observed on day 5-8 after hepatoma transplantation. Decreased AD activity was correlated with the inhibition of DNA synthesis. Hepatoma development was associated with progressive increase of purine nucleotide phosphorylase (PNP) activity in the thymus. There was a concomitant decrease of AD and PNP activities in the spleen RBC. Hepatoma growth was associated with marked (80%) inhibition of macrophage migration. These findings were indicative of the proliferation of the immunocompetent cells in the response to the tumor antigen. (28 Refs) (In RUS)

9. THE EFFECTS OF RETINOIC ACID AND A TUMOR PROMOTER, 12-O-TETRADECANOYLPHORBOL-13-ACETATE, ON LYMPHOCYTE PROLIFERATION.

Mastro AM, Pepin KG
Dept. Microbiology, Cell Biology and Biophysics, Pennsylvania State Univ., University Park, PA, 16802
Carcinogenesis; 3(4):409-413 1982

The tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibits DNA synthesis in allogenic, mixed cultures of bovine lymphocytes. Retinoic acid, an antagonist of TPA in in vivo skin promotion, was tested for its ability to counteract the effect of TPA on lymphocyte proliferation. Retinoic acid or related compounds, retinol or retinol acetate, did not reverse or prevent the inhibitory effect of TPA. Instead retinoic acid also inhibited DNA synthesis in mixed lymphocyte cultures. On the av, about 8 uM retinoic acid inhibited the mixed lymphocyte response by 50%. The mitogenic response of lymphocytes to phytohemagglutinin (PHA) was also inhibited by retinoic acid. The degree of inhibition depended both on the concentration of PHA and of retinoic acid. Therefore, in regard to bovine lymphocytes, retinoic acid depresses DNA synthesis in both allogenic and lectin stimulated responses. Such suppression should be taken into account in the use of retinoic acid in chemotherapy. (Author abstract) (29 Refs)

10. INDUCTION OF ALKALINE PHOSPHATASE AND THE GLYCOPROTEIN HORMONE ALPHA SUBUNIT IN HELA CELLS BY INHIBITORS OF DNA POLYMERASE.

Cox GS, Park KH
Dept. Biochemistry and Biophysics, Iowa State Univ., Ames, IA, 50011
Arch Biochem Biophys; 216(1):234-246 1982

Effects of two eukaryotic DNA polymerase inhibitors, aphidicolin (ApH) and phosphonoformic acid (PFA) on synthesis of placental alkaline phosphatase (PAP) and of the glycoprotein hormone alpha subunit in Hella cells were investigated. Both ApH (1.0-5.0 ug/ml) and PFA (1.2 or 3.8 milliM) inhibited DNA synthesis almost completely (82-93%), even at the lowest concentrations tested. Both inhibitors were good inducers of both PAP and the glycoprotein hormone alpha subunit. PAP activity was enhanced 60-100% by ApH at 1 ug/ml, and alpha subunit levels were increased 5-fold at an ApH concentration of 5 ug/ml. PAP activity showed a 7-to 8-fold increase and alpha subunit and a 2-to 3-fold increase as PFA concentration was increased to 3 milliM. Partial characterization of induced alpha subunit and PAP activity suggested that they are similar to the uninduced proteins. ApH (2.ug/ml) and PFA (2.5 milliM) acted synergistically with butyrate (3 milliM) but not with hydroxyurea (1 milliM). The fact that the concentration of ApH required to produce a half-maximal induction of the alpha subunit (15 ug/ml) was of an order of magnitude higher than the concentration required to inhibit DNA synthe-

sis by 50% (0.1 ug/ml) makes it unlikely that a direct relation exists between protein induction and DNA synthesis inhibition. (53 Refs)

11. EFFECT OF PULSE-PERIODIC LASER UV IRRADIATION OF LOW INTENSITY ON PROLIFERATING AND RESTING TUMOR CELLS.

Karu TI, Kalendo GS, Letokhov VS, Lobko VV
Res. Center Technological Lasers, Moscow, USSR
Dokl Akad Nauk SSSR; 262(6):1498-1501 1982

Irradiation of HeLa cells in the logarithmic growth phase (proliferating cells) or in the stationary phase (resting cells) with low a intensity pulse UV laser produced a reduction of DNA synthesis and permeability of cell membranes in proliferating cells. In the resting cells, pulse laser irradiation stimulated DNA synthesis and increased permeability of cell membranes for 3H-thymidine. Irradiation with continuous lasers inhibited DNA synthesis in proliferating cells but did not affect DNA synthesis in resting cells. (4 Refs) (In RUS)

12. ACTION SPECTRA FOR 8-METHOXYPSORALEN AND 3-CARBETHOXYPSORALEN IN SKIN FIBROBLASTS IN VITRO.

Christophers E, Schmidt B
Dept. Dermatology, Univ. Kiel, Schittenhelmstr. 7, D-2300 Kiel, W. Germany
Arch Dermatol Res; 272(3/4):245-250 1982

DNA synthesis was measured in guinea pig skin fibroblasts exposed to 8-methoxypsoralen (8-MOP: 0.01-10 ug/ml) or 3-carbethoxypsoralen (3-CP: 0.1-50 ug/ml) and UV (338.5-423 nanometers, 0.1 Joules/cm(2)). A linear decrease in photoinhibition of DNA synthesis with increasing wavelengths was seen after 8-MOP treatment, whereas 3-CP showed markedly reduced photoinhibition between 350 and 365 nanom. Results demonstrate that the UV action spectra should be considered when using furocoumarin derivatives in clinical practice. (23 Refs)

13. FURTHER STUDIES ON THE SURVIVAL OF NON-PROLIFERATING HUMAN DIPLOID FIBROBLASTS IRRADIATED WITH ULTRAVIOLET LIGHT.

Chan GL, Little JB
Dept. Pathology, Harvard Medical Sch. and Sidney Farber Cancer Inst., 44 Binney St., Boston, MA, 02115
Int J Radiat Biol; 41(4):359-368 1982

Labelling index data showed that in AG1518 cells, a diploid human fibroblast strain, there was a lag period of at least 14 hours between subculture from the density-inhibited plateau phase of growth and entry into DNA synthesis. Cells irradiated with 254 nm wavelength UV light 8 hr after subculture did not exhibit the same degree of resistance as cells irradiated in plateau phase and subcultured immediately. When cells were arrested from proliferation by maintenance in an arginine and glutamine deficient medium for 72 hr, they were nearly as resistant to UV light as plateau phase cells although maintenance in this medium for 24 hr after irradiation supported further recovery only after low UV doses. One strain of Cockayne syndrome fibroblasts was found to be resistant to UV light in plateau phase while another strain was found to have the same survival response whether it was irradiated in the plateau or log phase of growth. (Author abstract) (16 Refs)

14. PHORBOL ESTER INDUCTION OF LEUKEMIC CELL DIFFERENTIATION IS A MEMBRANE-MEDIATED PROCESS.

Cooper RA, Braunwald AD, Kuo AL
Hematology-Oncology Section, Dept. Medicine, Cancer Center, Hosp. Univ. Pennsylvania, Philadelphia, PA, 19104
Proc Natl Acad Sci USA; 79(9):2865-2869 1982

Phorbol esters are potent inducers of macrophage-like differentiation in the HL-60 promyelocytic leukemia cell line. The sequence of events by which they bring about this transition is poorly understood. However, it is known that phorbol esters bind to the surface membrane of HL-60 cells and to various other cells as well. Our studies were directed toward determining the biologic importance of this membrane association. [3H]Phorbol dibutyrate (PBu2) was specifically bound by HL-60 cells with a Kd of 23 nM and with 1.9 x 10(5) binding sites for [3H]PBu2 per cell. There was no internalization of bound [3H]PBu2. Specific binding was fully reversible upon washing in fresh medium, and [3H]PBu2 added thereafter bound normally to its receptor. Within 10 min of binding, PBu2 stimulated [14C]choline incorporation into phosphatidylcholine, with a rapid return to normal upon removal of the PBu2. Membrane-bound PBu2 progressively inhibited DNA synthesis, with 70% inhibition by 8 hr. This process was interrupted if the PBu2 was removed and little recovery of DNA synthesis occurred in previously inhibited cells. Between 8 and 16 hr, PBu2 induced adherence of cells to plastic, but only in those cells in which phosphatidylcholine synthesis was stimulated, and this process was also interrupted if PBu2 was removed prior to 16 hr. Similarly, nonspecific esterase, which develops after 72 hr of incubation, was induced in cells exposed to PBu2 for the initial 16 hr but not in cells exposed for 5 hr. These studies demonstrate that phorbol esters exert their effects while retained at the cell surface. Inhibition of cell growth and the acquisition of surface and enzymatic properties that characterize macrophages are separable events, each of which proceeds through a receptor-mediated, transmembrane process. The stimulation of phosphatidylcholine synthesis appears to be a part of that process. (Author abstract) (34 Refs)

15. IMMUNE STATUS OF UNTREATED PATIENTS WITH HODGKIN'S DISEASE AND PROGNOSIS.

Bjorkholm M, Wedelin C, Holm G, Ogenstad S, Johansson B, Mellstedt H
Dept. Medicine, Div. Hematology, Danderyd Hosp., S-182 88 Danderyd, Sweden
Cancer Treat Rep; 66(4):701-709 1982

In this study of 127 previously untreated adult patients with Hodgkin's disease and 167 age- and sex-matched controls, highly purified blood lymphocytes were studied for E+ receptors; their spontaneous DNA synthesis and that induced by concanavalin A, pokeweed mitogen, or purified protein derivative of tuberculin were measured as incorporation of 14C-thymidine. T-cell counts and the response to mitogens and antigen were significantly decreased in the patients, while the spontaneous lymphocyte activity was increased. Forty-nine patients had died after a mean observation time of 6 yr. In these patients, there had been an increase in spontaneous DNA synthesis and a decrease in that induced by mitogens and antigen. These differences also remained significant when patients who died were compared with surviving patients in Stages I-II. The 5-yr survival rate of 'bad immunology' patients was 30% as compared to 80% for the remainder. We suggest that these lymphocyte tests should be considered in an 'immunologic staging system,' which may give guidance in the future man-

agement of patients with Hodgkin's disease. (Author abstract) (25 Refs)

16. DISSOCIATION BETWEEN TUMOUR CELL CYTOSTATIC AND IMMUNO-SUPPRESSIVE CAPACITY OF HUMAN MONOCYTES ACTIVATED WITH LYMPHOKINES.

Unsgaard G, Hammerstrom J, Lamvik J
Trondheim Regional Hosp., N-7000 Trondheim, Norway
Acta Pathol Microbiol Scand [C]; 90(2):73-79 1982

Activation of monocytes with lymphokines from BCG-stimulated lymphocytes increased the capacity to inhibit DNA-synthesis in a tumor cell line (NHIK 3025) at all stages of monocyte maturation in vitro. However, the capacity of monocytes to suppress DNA-synthesis in mixed lymphocyte cultures (MLC) was reduced, indicating a dissociation of the immunosuppressive and tumor cell cytostatic functions of lymphokine-activated monocytes. Supernatants of lymphokine-activated monocytes inhibited DNA synthesis in the tumor cell line, but not in MLC. Monocytes from donors with a strong tuberculin reaction produced supernatants with a stronger cytostatic activity than monocytes from donors with a weak tuberculin reaction. By in vitro maturation, even the latter monocytes acquired ability to produce supernatants that were strongly inhibitory to tumor cells. (Author abstract) (23 Refs)

17. EVIDENCE SUGGESTING THAT THE MECHANISM FOR AEROBIC AND HYPOXIC CYTOTOXICITY OF NITROHETEROCYCLES IS THE SAME.

Olive PL
Section Radiobiology, Johns Hopkins Oncology Center, 600 North Wolfe St., Baltimore, MD, 21205
Int J Radiat Oncol Biol Phys; 8(3/4):687-691 1982

The fluorescence of three nitroheterocycles was used to measure the uptake and binding of the compounds by cells under hypoxic and aerobic conditions. Chinese hamster V-79 and mouse L-929 cells were exposed in suspension cultures to 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), trans-5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,4 oxadiazole (AVO), or 4-nitroquinoline-1-oxide (4-NQO). Fluorescence was measured in single-cell samples by means of a fluorescence-activated cell sorter. Cellular fluorescence correlated with cell survival independently of the concentration of AF-2, time of exposure, or cell density, indicating that cell death was dependent on the amount of AF-2 taken in and bound by the cell. Higher concentrations of AF-2 in the medium were required for cell killing under aerobic conditions than under hypoxic conditions. However, at concentrations that produced equal toxicity, cellular fluorescence was similar under hypoxic and aerobic conditions. A correlation between cellular fluorescence and cell survival was also shown for 4-NQO. Incubation of V-79 cells with glutathione produced reductions in both toxicity and cellular fluorescence. Inhibition of DNA synthesis by AF-2 was independent of oxygen tension. Results suggest that the mechanism involved in cytotoxicity is the same under hypoxic and aerobic conditions. Oxygen evidently affects the rate of uptake of nitroheterocycles, rather than the nature of the toxic species. The toxic species is most likely the nitro anion radical. (18 Refs)

18. ORNITHINE DECARBOXYLASE INDUCTION AND THE CYTOSKELETON IN NORMAL AND TRANSFORMED CELLS.

Rumsby G, Puck TT
(c/o Puck) Eleanor Roosevelt Inst. Cancer Res., Univ. Colorado Health Science Center, Denver, CO, 80262
J Cell Physiol; 111(2):133-139 1982

Ornithine decarboxylase (ODC) induction of normal human skin fibroblasts by colchicine (COL) and other cytoskeletal disorganizing agents was compared to that of HeLa and transformed CHO-K1 cells. Treatment with fresh medium alone was sufficient to release a large fraction of the G1 block in HeLa cells, whereas serum was required to release normal fibroblasts from G1 block. DNA synthesis in the growth arrested cells was minimal and release from G1 block was followed by initiation of ODC synthesis and subsequently of DNA synthesis in both cell lines. Induction of ODC by medium change was completely prevented in both cell lines by addition of 10 ug/ml actinomycin D (ACTD) or 25 ug/m cycloheximide (CYH). The fact that ODC activity in cells released from G1 block revealed rising and falling phases indicated that both activating and inactivating processes were involved. While increasing COL concentrations ODC induction decreased in normal fibroblasts. The curve for HeLa cells was biphasic. Vinblastine produced effects similar to those of COL. If inhibitory drugs were not added until several hr after medium change, CYH alone produced a steadily diminishing ODC activity in both cell lines. In the absence of protein synthesis, COL did not contribute any additional effect to the enzyme inactivation process. The inhibitory action of COL was probably exerted on prevention of protein synthesis rather than inactivation of already synthesized enzyme. The CHO-K1 cell line resembled HeLa cells in all respects. (30 Refs)

19. CLINICAL AND CELL KINETIC STUDIES OF THE EFFECTS OF THE EPIPODOPHYLLOTOXIN VP16-213 DURING THERAPY OF REFRACTORY ACUTE NONLYMPHOCYTIC LEUKEMIA.

Look AT, Dahl GV, Rivera G, Mauer AM
Div. Hematology-Oncology, St. Jude Children's Res. Hosp., 332 N. Lauderdale, P.O. Box 318, Memphis, TN, 38101
Cancer Chemother Pharmacol; 7(2/3):161-167 1982

The efficacy of two new drugs, VP16-213 and 5-azacytidine (5AZ), both effective single agents for therapy of refractory acute nonlymphocytic leukemia (ANLL), was tested in 24 patients (11 male, 13 female; 1-19 yr old), who had induction failure (5) or were in relapse (19). The treatment schedule was 200 mg VP16-213/square meter (m2) iv daily x 3 days followed by 300 mg 5AZ/m2 iv daily X 2 days. Until bone marrow hypoplasia was found by marrow aspiration, the schedule was repeated every 1-2 days. Nineteen of 24 patients had marrow hypoplasia and 11/24 achieved complete remission; of the latter 11, 5 remained in remission for 12-29 wk, demonstrating a highly effective drug combination. The effect of VP16-213 on leukemia blast proliferation was studied using patient serial bone marrow aspiration and DNA histogram analysis. G2 phase cell cycle arrest was found in leukemic blasts of all eight patients studied 4 and 24 hr after initial VP16-213 administration. DNA histogram analysis indicated a decrease in the number of cells in DNA synthesis after three daily doses of VP16-213. Such an occurrence is probably due to selective killing of cells in the S-phase by the drug compared to those in the G0/G1 phase. (18 Refs)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

20. CHANGES IN DNA-POLYMERASE AND THYMIDINE KINASE ACTIVITY DURING INTERFERON TREATMENT.

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Biochem Biophys Res Commun; 105(4):1569-1576 1982

The effect of human interferon beta (IFN-beta) on the human glioma cell line, U-251 MG, was investigated. Exponentially growing cells treated with IFN exhibited a dose-dependent growth depression observed after 3 days, and an increase in the amount of cells in S phase. However, the activity of DNA polymerase was decreased to 30% of control levels at day 5 with 1,000 Units/ml of IFN-beta. This depression in enzyme activity may be explained by an inhibitory effect of IFN on the increase of enzyme activities induced by events in late G1 or early S, or by the induction of diffusable factors depressing enzyme activities. Starved cells were induced to cycle and DNA content was followed cytofluorometrically. S phase started approx 12 hr after addition of serum to synchronized cells and cells entered G2 at 21 hr. In contrast, IFN-treated cultures initiated S but accumulated in that phase. At the start of S phase, both DNA polymerase and thymidine kinase activity increased, although the rate of increase was markedly reduced by treatment with IFN. No inhibitory factor could be identified in the IFN treated cultures. It is probable that IFN has a direct effect on the induction of the studied enzyme activities, thus affecting virus replication. (18 Refs)

21. DIFFERENTIAL INHIBITION OF DNA POLYMERASE AND RNASE H ACTIVITIES OF THE REVERSE TRANSCRIPTASE BY PHOSPHONOFORMATE.

Margalith M, Falk H, Panet A
Dept. Virology, Hebrew Univ., Hadassah Medical Sch., Jerusalem, Israel
Mol Cell Biochem; 43(2):97-103 1982

Three potential inhibitors of reverse transcriptase activities, phosphonoformate (PF), phosphonoacetate (PAA), and ethyldiethyl phosphonoformate (Et-PF), were compared in this study. Only PF was found to inhibit the DNA polymerase activity of the purified reverse transcriptase of Moloney murine leukemia virus (M-MuLV) and avian myeloblastosis virus (AMV). The degree of DNA polymerase inhibition was linear with PF concentration; 50% inhibition was achieved at 10 uM. Whereas PF inhibited both the RNA and DNA dependent DNA polymerase activities, the RNase H activity of the reverse transcriptase was unaffected. Both the endogenous DNA polymerase activity in detergent disrupted virus and the activity of the purified enzyme with the isolated virus genome 70S RNA were inhibited by PF. However, higher concentrations of PF were needed to inhibit the endogenous reaction. The inhibition by PF appeared to be reversible and noncompetitive with respect to the substrate deoxythymidine triphosphate (dTTP). Addition of PF after the initiation of DNA synthesis immediately arrested the reaction. (Author abstract) (26 Refs)

22. ACTIVITIES OF VARIOUS ENZYMES OF PYRIMIDINE NUCLEOTIDE AND DNA SYNTHESES IN NORMAL AND NEOPLASTIC HUMAN TISSUES.

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Gan; 73(2):289-298 1982

The activities of the key enzymes of pyrimidine nucleotide and DNA syntheses in 43 human tumors and 28 normal human tissues were investigated. The activities of cytidine triphosphate synthetase, deoxycytidine monophosphate deaminase, uridine kinase, thymidine kinase, thymidine monophosphate kinase and DNA polymerase were markedly increased in tumor tissues, compared with those in the corresponding normal tissues, while the activities of deoxycytidine kinase, cytidine deaminase and deoxycytidine deaminase were only slightly increased. The use of thymidine and deoxyuridine as substrates of human pyrimidine nucleoside phosphorylase gave 1 to 2 orders of magnitude higher activity than that of uridine. (Author abstract) (30 Refs)

23. IMMUNOCYTOCHEMICAL METHOD FOR THE DETECTION OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE IN ACUTE LEUKEMIA.

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Klin Wochenschr; 60(9):451-455 1982

Terminal deoxynucleotidyl transferase (TdT) has become an important marker for the classification of acute leukemias. Originally detected by a biochemical assay, the presence of this enzyme in leukemia can now be detected by making use of a highly specific antibody and by applying the immunofluorescence method. In this study, an attempt was made to apply the unlabeled peroxidase-antiperoxidase (PAP) technique for the examination of TDT positive cells. We have optimized this method and have compared the different assays - biochemical, immunofluorescence, and immunocytochemical (PAP) - in 10 patients with acute leukemia. The results obtained from all three methods are in accordance with one another, but the PAP method is a further simplification for the detection of TdT in the routine diagnosis of acute leukemias. (Author abstract) (13 Refs)

24. ENRICHMENT OF CELL POPULATIONS CONTAINING TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE ACTIVITY BY FREE FLOW ELECTROPHORESIS.

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Int J Cancer; 29(5):501-506 1982

Terminal deoxynucleotidyl transferase (TdT) is an enzyme that has been utilized as a biochemical marker for following certain leukemia patients during treatment and remission. The potential usefulness of this enzyme in predicting the onset of relapse before any morphological indications has been demonstrated in chronic myelogenous leukemia patients in blast phase of the disease. In order to be able to detect low levels of TdT activity, especially during remission phase, we have used the technique of free flow electrophoresis to enrich cell populations containing TdT. With this technique we have achieved up to 20-fold enrichment of TdT-positive cell populations from

normal human bone marrow, cells from patients with acute lymphoblastic leukemia and chronic myelogenous leukemia in blast phase of the disease. TdT positive cells were not detected in patients with acute myelogenous leukemia and B cell lines. This technique should prove to be useful in early detection of relapse in patients in remission and for enrichment of cell populations containing other biochemical markers. (Author abstract) (22 Refs)

MECHANISMS OF DNA REPAIR

25. RECENT DEVELOPMENTS IN THE MECHANISMS OF DNA REPAIR AND IN THE ROLE OF DNA REPAIR IN CHEMICAL CARCINOGENESIS AND CANCER CHEMOTHERAPY.

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Adv Exp Med Biol; 136(partB):1367-1387 1982

Mechanisms of DNA repair, including excision repair, and the role of DNA repair in chemical carcinogenesis and cancer chemotherapy are discussed. Base excision (small patch repair) and nucleotide excision (large patch repair) are described and a two-step model for excision repair of pyrimidine dimers is illustrated. Whether any specific excision repair enzyme acts similarly on UV- and chemically-induced DNA damage to remove a modified base by glycosylase action is unproven. Inducible excision repair processes in prokaryotic cells (adaptation and SOS repair) and in mammalian cells is discussed. Reactivity of compounds can be correlated with mutagenicity and carcinogenicity: agents that react by a unimolecular substitution reaction (SN1) are the most potent mutagens and the most potent carcinogens. SN1 reactivity in turn correlates with the ability of compounds to react more extensively with the oxygen atom sites in DNA. The physiological state of a tissue determines not only the degree of cell proliferation taking place, but also the cytotoxic effect of DNA lesions that modify or act as a block to DNA replication. DNA excision repair and cytotoxicity is discussed. Normal cells may be proficient in repair of alkylation damage, while tumor strains may be either proficient or deficient in repair. Replication of DNA on a damaged template (RDT), or post-replication repair, occurs when unexcised DNA damage is circumvented during replication. In rodent cells, RDT may be adversely influenced by post-treatment incubation in the presence of caffeine. RDT may occur by gapped synthesis or replicative bypass (branch migration). Base mispairing and the introduction of potentially mutagenic lesions has been implicated in the etiology of nitrosamine-induced carcinogenesis. (68 Refs)

26. TIME-SCALE AND NATURE OF RADIATION-BIOLOGICAL DAMAGE: APPROACHES TO RADIATION PROTECTION AND POST-IRRADIATION THERAPY.

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Prog Biophys Mol Biol; 39(2):69-107 1982

Time-scale and effects of energy deposition by high energy radiation applied to biological systems are reviewed, and the consequences of DNA, protein, cellular, mitotic, and carcinogenic damage are discussed. The nature of DNA repair and the roles of hydroxyl radicals, superoxide anions, and singlet oxygen in inducing biological damage are reviewed. Radiation protection and post-irradiation therapy are discussed at the biochemical level. (400 Refs)

27. ULTRAVIOLET RADIATION: INTERACTION WITH BIOLOGICAL MOLECULES.

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Cancer - A Comprehensive Treatise Vol. 1. Etiology - Chemical and Physical Carcinogenesis. Second Edition. Becker FF, ed. New York, Plenum Press, 714 pp., 1982.

Among the major effects of UV radiation on biological systems are inhibition of cell division, inactivation of enzymes, induction of mutation, and killing of cells and tissues. Some of the biological effects of UV radiation can now be explained in terms of specific chemical and physical changes produced in DNA. Structural defects are produced in DNA by chemical mutagens and by radiations. Three major kinds of DNA recovery have been described. The damaged molecule can be restored to its functional state in situ, or it may be removed and replaced with undamaged material to restore normal function, or it may remain unrepaired, but the cell may either bypass or ignore the damage. Enzyme-catalyzed photoreaction is the best-known form of in situ repair. Excision repair involves recognition, incision, excision and repair replication, and rejoining. Several observations and biological and biochemical studies support the assumption that exposure to sunlight, particularly the mid-UV component, is an essential and major factor in the development of most skin cancers. Skin cancer appears on those parts of the body most exposed to sunlight, particularly the head, neck, arms, and hands. (54 Refs)

28. EUKARYOTIC DNA REPAIR IS BLOCKED AT DIFFERENT STEPS BY INHIBITORS OF DNA TOPOISOMERASES AND OF DNA POLYMERASES ALPHA AND BETA.

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Biochim Biophys Acta; 697(1):6-13 1982

Inhibitors of (a) DNA topoisomerases (novobiocin and nalidixic acid) and of (b) eukaryotic DNA polymerses alpha (cytosine arabinoside) and beta (dideoxythymidine) blocked different steps of DNA repair, demonstrated by the effects of the inhibitors on the relaxation of supercoiled DNA nucleoids following treatment of human cell cultures with ultraviolet light (1-3 J/m2) or N-methyl-N-nitro-N-nitrosoguanine MNNG (5 or 20 uM) and the subsequent restoration of the supercoiled nucleoids during repair incubation. Changes in the supercoiling of nucleoid DNA were assayed by analysis of their sedimentation profiles in 15-30% neutral sucrose gradients. Inhibition of repair by novobiocin was partially reversible; upon its removal from the culture medium, the nucleoid DNA of repairing cells became relaxed. The DNA polymerse inhibitors allowed the initial relaxation of DNA after treatment of the cells with UV or MNNG but delayed the regeneration of the initial rapidly-sedimenting (supercoiled) nucleoid DNA for 2-4 hr. Dideoxythymidine (1 mM) was more effective than cytosine arabinoside (1 uM) in producing this delay, but neither inhibitor by itself blocked repair permanently. Incubation of ultraviolet-irradiated cells with 1 uM cytosine arabinoside plus 1 mM dideoxythymidine blocked the completion of repair for 24 hr, whereas incubation with 10 uM cytosine arabinoside or 5 mM di-

deoxythymidine produced only temporary repair delays of 2-4 hr. Thus, it is likely that the two DNA polymerase inhibitors act upon separate targets and that both targets are involved in repair. It is concluded from these and from previous studies that (1) the DNA repair-sensitive target of novobiocin and nalidixic acid in vivo is not a DNA polymerase, but, rather, a DNA topoisomerase; (2) this target affects an initial step of DNA repair leading to the relaxation of supercoiled DNA; (3) the DNA polymerization step of repair may involve both alpha- and beta-type DNA polymerases; and (4) in repair one type of DNA polymerase may substitute for another. (Author abstract) (33 Refs)

29. THE EFFECT OF MISONIDAZOLE ON THE CYTOTOXICITY AND REPAIR OF POTENTIALLY LETHAL DAMAGE FROM ALKYLATING AGENTS IN VITRO.

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Int J Radiat Oncol Biol Phys; 8(3/4):761-765 1982

The survival of the plateau phase Chinese hamster ovary HA-1 cells in vitro after aerobic exposure to various alkylating agents was examined. The determination of hypoxia presence for the inhibition by misonidazole (MISO) of the recovery from potentially lethal damage (PLD) due to alkylating agents and for MISO chemosensitization was studied. The presence of 5 milliM MISO during the 1-hr melphalan (L-PAM) incubation period enhanced the cell killing by the alkylating agent. If the cells were returned to the culture conditions for a 24-hr period after exposure to the drugs, before survival was assayed they were able to repair some of the L-PAM-induced PLD. MISO caused a similar degree of enhancement of L-PAM toxicity towards the cells. Similar results were observed for cyclophosphamide. PLD recovery also was observed when HA-1 cells were exposed to the monofunctional alkylating agent, methyl methanesulfonate (MMS). However, incubation of the cells with MISO during the period they were in contact with MMS had no additional effect on the toxicity. The slow elution rate for L-PAM compared to irradiated controls indicated that DNA-crosslinks were being formed. MMS toxicity appeared to be the result of the formation of a single strand DNA breaks. MISO had no effect on L-PAM- or MMS-induced DNA damage. For L-PAM, a post-incubation in MISO, even for 24 hr seemed to have little effect in increasing the cell killing seen when MISO was present during the 1-hr drug exposure period. However, with MMS, post-incubation in MISO significantly enhanced toxicity. (11 Refs)

30. MECHANISM BY WHICH CAFFEINE POTENTIATES LETHALITY OF NITROGEN MUSTARD.

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Proc Natl Acad Sci USA; 79(9):2942-2946 1982

Caffeine is synergistic with many DNA-damaging agents in increasing lethality to mammalian cells. The mechanism is not well understood. Our results show that caffeine potentiates the lethality of the nitrogen mustard 2-chloro-N-(2-chloroethyl)-N-methylethanamine (HN2) by inducing damaged cells to undergo mitosis before properly repairing lesions in their DNA. Treatment with low doses of HN2 (0.5 uM for 1 hr) caused little lethality in baby hamster kidney cells (90% survival). These cells were arrested in G2 shortly after treatment with HN2 as shown by flow microfluorimetry and autoradiography. After an arrest of 6 hr, HN2-treated cells began to move into mitosis and from then on behaved like normal cells. Repair synthesis was shown to continue during the G2 arrest by using synchronized cells pulse labeled with [3H]thymidine after HN2 treatment and autoradiography. Caffeine (2 mM) increased the lethality of HN2 by 5-to 10-fold. It prevented the G2 arrest. Caffeine did not prevent these HN2-treated cells from entering or completing S phase but rather allowed them to divide without finishing the repair processes and as a consequence caused nuclear fragmentation after mitosis. Caffeine-induced nuclear fragmentation and enhanced lethality were proportional, as shown with dose-response curves and time dependence. In addition, both lethality and nuclear fragmentation were abolished by low doses of cycloheximide, an inhibitor of protein synthesis. (Author abstract) (34 Refs)

31. STRUCTURAL CHANGES IN MAMMALIAN CELL DNA INDUCED BY LOW-DOSE X-RAY DAMAGE AND SUBSEQUENT POSTIRRADIATION INCUBATION IN THE PRESENCE AND ABSENCE OF CAFFEINE.

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Radiat Res; 90(2):310-320 1982

Experiments on low-dose (30-2,000 rad) x-ray-induced damage in rat 9L cells in vitro and subsequent posttreatment irradiation measured under neutral (pH 7) conditions are described. Results indicated that the principal retardation time of DNA in neutral lysates was sensitive to x-irradiation of the cells. The retardation time increased by about a factor of seven in the dose range of 0-1,000 rad, the major portion of the increase occurring with the first 300 rad. The biphasic nature of the response to increasing doses of radiation appeared to be unique to the viscoelastic method utilized. The viscoelastic retardation time showed a complicated pattern reflecting different structures of DNA during postirradiation incubation. No indication of loss of large DNA during postirradiation incubation was observed. Results were interpreted as suggesting conformational changes in damaged DNA during repair. Post-irradiation incubation in the presence of 0.5 milliM caffeine resulted in inhibition of DNA repair. (29 Refs)

32. REPAIR OF DNA INTERSTRAND CROSSLINKS AFTER BUSULPHAN. A POSSIBLE MODE OF RESISTANCE.

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Cancer Chemother Pharmacol; 8(1):3-7 1982

The technique of alkaline elution was used to study the interaction between busulfan (BS) and the DNA of cells derived from the transplantable rodent Yoshida sarcoma. The findings were related to the in vitro cytotoxicity and chromosome aberrations (CHA) in the anaphase of cell lines resistant (YR) and sensitive (YS) to BS are discussed. The doses of BS producing 50% cell death were 7.5 and 54.5 ug/ml for YS and YR, respectively. BS (50 ug/ml) treatment in both cell lines caused a greater than two-fold depression in the mitotic index after 24 hr post-treatment incubation. A four-fold greater number of CHA could be observed in the YS than the YR line. Typical elution profiles in YS cells after a single 1-hr dose of BS (100 ug/ml) are presented. A small but reproducible proteinase-resistant retention was observed immediately after BS treatment, indicating the presence of DNA interstrand cross-links. As the dose of BS was increased, an increasing filter retention was ob-

served, from which the number of cross-links per unit DNA could be assessed. The relationship between drug dose and the frequency of DNA interstrand cross-links is shown. A temporal study of DNA interstrand cross-linking after a single dose of BS (50 ug/ml; 1 hr) revealed a gradual increase in cross-linking over the first 4 hr, followed by a disappearance over 24 hr in the YR line. In the YS line, an increase in cross-linking over 4 hr and very little loss was observed 24 hr after drug removal. The time-course of cross-linking in both lines in terms of the relative retention is shown. (19 Refs)

33. INTERACTION OF VP16-213 WITH THE DNA REPAIR ANTAGONIST CHLOROQUINE.

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Cancer Chemother Pharmacol; 7(2/3):123-126 1982

A semi-synthetic derivative of podophyllotoxin, VP16-213, has significant antitumor activity and is able to produce single strand DNA breaks in HeLa cells. Chloroquine (CHL), a 4 amino quinalone antimalarial drug, inhibits the repair of single strand breaks caused by methyl methane sulfonate in cultured rat liver cells. The synergistic kinetics of these drugs were studied in a murine lymphoma model (TLX5 murine ascites). Acute and chronic toxicity studies indicated additive toxicity, as manifested by seizures and marked weight loss. Varying the timing of CHL administration affected the mean percent increased life span (ILS). When CHL was administered following VP16-213, no improvement in ILS occurred, but when administered simultaneously, a markedly increased ILS was found. CHL alone had no significant antitumor activity against TLX5 lymphoma. Vincristine, which also showed antitumor activity against TLX5 lymphoma, showed no enhanced effect when used in combination with CHL. VP16-213 is beginning Phase III studies but due to toxicity of CHL in man, this combination will probably not be used clinically. (20 Refs)

34. REACTIVE METABOLITES AS KEY INTERMEDIATES IN PHARMACOLOGIC AND TOXICOLOGIC RESPONSES: EXAMPLES FROM CHEMICAL CARCINOGENESIS.

Miller EC, Miller JA
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Adv Exp Med Biol; 136(partA):1-21 1982

Electrophilic reactants as toxic agents, mechanism of chemical carcinogenesis, and metabolic activation of chemical carcinogens (CC) and of 1'-hydroxyestragole (HE) are discussed. Current data on carcinogenesis indicate that the first stage, initiation, depends on the reaction of a strong electrophilic reactant (ER) with DNA and a resultant mutation of the DNA. The second stage, promotion, requires a prolonged time for completion and appears to be reversible in the early phases. The activation reactions of critical importance in the induction of tumors are influenced by the many factors (molecular geometry, polarity) that determine the approach of the procarcinogen or proximate carcinogen to the enzyme(s), the properties of the enzyme(s) in relation to the particular substrate, and the susceptibility of ER to inactivating reactions before they reach the critical target for the induction of neoplasia. Examples of some reactions for the activation of procarcinogens to ER are summarized and the metabolic activation of a broader range of CC is shown. The characterization of the DNA adducts formed from HE was studied in mouse liver. Co-chromatography in three system showed the apparent identity of the principal products formed in vivo with the principal products formed by reaction of 1'-acetoxyestragole with deoxyguanosine (3 products) and deoxyadenosine (1 product). Data suggested that each of the deoxyguanosine adducts was substituted on the exocyclic amino group. The mutagenic and carcinogenic responses of cells to the attack by chemicals depend on the genes in the DNA that are affected, the susceptibility of the modified nucleic acid residues to repair, the fidelity of the repair, and the effects of unrepaired lesions on DNA replication. (56 Refs)

35. CYTOLOGIC AND BIOCHEMICAL GENETIC EFFECTS OF CHEMICAL CARCINOGENS. ANNUAL REPORT, 1 JUL. 1980-30 JUN. 1981.

Rasmussen RE
Ohio Aerospace Medical Res. Lab., Wright-Patterson Air Force Base, OH
Sci Tech Aerosp Rep; 20(8):1128 1982

The first project is concerned with the relationship between initiation of cancer by chemicals and the damage and repair of DNA in cells and tissues susceptible to the carcinogenic action of the chemicals. The working hypothesis is that interaction of the carcinogen with cellular DNA is a necessary step in carcinogenesis and that this interaction may cause DNA damage which is then followed by DNA repair synthesis. The second project was conducted with the cooperation of the staff of the Toxic Hazards Research Unit at AMRL, WPAFB. Rats and hamsters were exposed by inhalation to hydrazine at 750 ppm for 1 hr. Groups of animals which had been exposed only once or which had been exposed 10x were shipped to UC Irvine for study. The factors studied were (1) DNA replication in the lung; (2) DNA repair synthesis in the lung; (3) Cellular replication in the lung and nasal turbinates; and (4) Metabolism of a known lung carcinogen, 3H-benzo(a)pyrene, by lung slices from the treated animals. The third project was initiated during the current year with the goal of developing an animal model in which biochemical and cytogenetic factors could be studied during the process of tumorigenesis. Range-finding studies are underway at the Toxic Hazards Research Unit (THRU) to establish the intratracheal dosage of 3-methylcholanthrene (MCA) necessary to produce a high yield of lung tumors in Fischer 344 rats within 12 mo. (Author abstract)

36. ANTIMUTAGENIC EFFECT OF ISOCYANATES AND RELATED COMPOUNDS IN ESCHERICHIA COLI.

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Gan; 73(2):255-263 1982

Isocyanates and isothiocyanates have been suggested to inactivate enzymes involved in the metabolic activation of chemical carcinogens and the repair of DNA damage. These compounds decrease the mutability of a tester strain of Escherichia coli B under UV irradiation. This paper deals with the antimutagenicity of acylating agents, including isocyanates and isothiocyanates, and some anti-oxidants which are suspected to be anticarcinogenic. The results can be summarized as follows. (1) The antimutagenic effect observed in the present study operates on UV-induced mutagenesis but not on x-ray-induced mutagenesis. (2) This effect operates only on the wild-type strain, H/r30R, but not on Hs30R deficient in the excision repair system. (3) This effect may function through giving the irradiated cells a greater chance to carry out excision repair by prolonging the lag-period before entry into the S-phase. (4) The carbomoylating ability of isocyanates and isothiocyanates may be responsible for the antimutagenicity but other type of reactivities may also be involved. These antimutagens also partici-

pate in inactivating enzymes relevant to the metabolic activation of mutagens, resulting in a decrease in the frequency of chemically induced mutagenesis. (Author abstract) (30 Refs)

37. ROLE OF DNA REPAIR IN ETHYL METHANESULFONATE-INDUCED MUTAGENESIS IN SACCHAROMYCES CEREVISIAE.

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Dept.Radiation Biology and Biophysics, Univ. Rochester Sch. Medicine, Rochester, NY, 14642
Carcinogenesis; 3(4):439-444 1982

Ethyl methanesulfonate (EMS)-induced reversion of two sites which revert preferentially by GC to AT transitions, cycl-131 and cycl-115, has been examined in radiation sensitive, rad, mutants of yeast belonging to the rad52 epistasis group. The rad50, rad51, rad52, rad54 and rad56 mutants showed reduced reversion of both tester sites when stationary phase diploid cells were treated with EMS. No correlation was found between EMS-induced reversion and EMS-induced homologous mitotic intragenic recombination. Survival of rad6 rad52 double mutants following EMS treatment indicates that there is one epistasis group for the repair of EMS-induced lethal damage in yeast. A model involving misrepair mutagenesis of specific lesions is proposed to account for the experimental results. (Author abstract) (39 Refs)

38. KINETICS OF REPAIR OF DNA SINGLE-STRAND BREAKS IN CULTURED MAMMALIAN CELLS. PART I. THE DEPENDENCE ON IRRADIATION DOSE AND CELL CYCLE STAGE.

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Int J Radiat Biol; 41(4):453-457 1982

Kinetics of repair of DNA single-strand breaks were examined in asynchronous cultures of Chinese hamster cells and in cells synchronized at the G1 stage after irradiation with doses of 15 and 200 grays (Gy), which induce reproductive and interphase death of cells, respectively. Irradiation led to practically the same DNA degradation independent of cell cycle stage. In all experiments with asynchronous cultures, repair started immediately after irradiation with 15 and 200 Gy and did not depend on the pH of the medium in the range 6.5-8.0. The major part of the strand breaks repaired quickly enough. Following exposure to 15 Gy, the repair proceeded at a constant rate in both cultures; however, the repair rate was twice as low in synchronous as in asynchronous cultures. After irradiation with 200 Gy, the repair processes in both cultures started with the rates close to those at a lower dose. However, 5 min later, the repair rates became about 5-6x lower in asynchronous cultures; in asynchronous cultures, it was 2x as low as that in asynchronous cultures. Then the rates remained practically constant in both cultures. (14 Refs)

39. FIBRE AUTORADIOGRAPHY OF REPAIR AND REPLICATION IN DNA FROM SINGLE CELLS: THE EFFECT OF DNA SYNTHESIS INHIBITORS.

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Eur J Cell Biol; 27(1):25-33 1982

DNA fiber autoradiography, after incorporation of high specific activity 3H-thymidine and 3H-deoxycytidine, has been used to investigate repair in DNA fibers from single cells following UV, or methyl-methane sulfonate (MMS) treatment. Asynchronously growing human fibroblasts, leukocytes, and HeLa cells at different phases of the cell cycle have been investigated. Isotope incorporation in repair could be differentiated from that involved in replication by the distribution and density of silver grains along the DNA fibers. Grain distribution due to repair was continuous over long stretches of the fibers and was at a low density, occasionally interspersed with short slightly denser segments. Replication labeling on the other hand, was dense and usually in short tandem segments. Repair labeling was of a similar overall density in fibers from a single cell, but differed in intensity from cell to cell. In mutagen treated Go (leukocytes) or G1 (HeLa cells), repair labeling was not increased by the presence of the DNA inhibitors, hydroxyurea (HU) or 5-fluorodeoxyuridine (FUdR). Repair was not detectable in S cells however without the use of these inhibitors to reduce endogenous nucleoside production. FUdR enhanced the repair labeling in S cells only slightly, while HU increased it beyond that observed in UV irradiated, HU treated, G1 cells. The intensity of repair labeling in fibers from mutagen treated S cells appears to be proportional to the degree of reduction of DNA chain elongation in replicons. (Author abstract) (23 Refs)

40. VISCOSIMETRIC ANALYSIS OF THE OCCURRENCE AND REPAIR OF DNA SINGLE-STRAND BREAKS IN IRRADIATED ANIMAL TISSUES.

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Int J Radiat Biol; 41(4):389-399 1982

The yields of immediate DNA single-strand breaks in normal tumor tissues of irradiated animals were measured by a viscosimetric method of determination of high-polymer single-strand DNA molecular weight in alkaline nuclear lysates. It has been shown that in irradiated thymus, bone marrow leukocytes, Ehrlich ascitic carcinoma and Zaidel hepatoma cells (1st group of tissues) in vivo the yields of DNA single-strand breaks were characterized by 80 to 130 eV per break. In in vivo irradiated liver, lymph node, spleen, and sarcoma 180 cells (2nd group of tissues) the yields of DNA single-strand breaks have been characterized by 30 to 40 eV per break. DNA single-strand breaks of the first group of tissues have joined 1 hr after the irradiation in vivo; DNA single-strand breaks of the second group have not done so. (Author abstract) (27 Refs)

41. ADP-RIBOSYLTRANSFERASE ACTIVITY IN CULTURED HEPATOCYTES. INTERACTIONS WITH DNA REPAIR.

Althaus FR, Lawrence SD, Sattler GL, Pitot HC
McArdle Lab. for Cancer Res., Univ. Wisconsin Medical Sch., Madison, WI, 53706
J Biol Chem; 257(10):5528-5535 1982

A rapid increase in ADP-ribosyltransferase activity was observed when freshly isolated hepatocytes derived from adult rats were established in primary monolayer culture. (ADP-ribose)n-degrading activity remained constant over a period of 48 hr of culture. Inhibition of ADP-ribosyltransferase activity with pyridine derivatives, 3-aminobenzamide, theophylline, or thymidine, was accompanied by an enhanced DNA repair synthesis in response to the direct-acting carcinogen, methyl methanesulfonate, or UV irradiation. Three aminobenzamides differing only in the position of the amino group exhibited the same structure-activity relationship in regard to their action on

DNA repair synthesis and ADP-ribosyltransferase. Spermine treatment of hepatocytes apparently had an inverse effect on both these cellular functions. The removal of DNA strand breaks following methyl methanesulfonate treatment was accelerated by inhibitors of ADP-ribosyltransferase. The results suggest that ADP-ribosylation interacts with late stages in the process of DNA repair. This interaction apparently is dependent on the nature of damage imposed on chromatin since repair synthesis in response to a number of carcinogens is unaffected by inhibitors of ADP-ribosyltransferase. (Author abstract) (52 Refs)

42. PLASMID R46-MEDIATED PROTECTION AGAINST BLEOMYCIN IS POLA+-DEPENDENT.

Attfield PV, Pinney RJ
Microbiology Section, Dept. Pharmaceutics, Sch. Pharmacy, Univ. London, Brunswick Square, London WC1N 1AX, England
J Gen Microbiol; 128(part3):539-547 1982

Strains of Escherichia coli deficient in post-replication recombination repair were more sensitive to bleomycin than wild-type, repair-proficient strains. Mutants lacking excision repair functions were no more sensitive to bleomycin than the wild-type strains, indicating that this pathway is not involved in the repair of bleomycin-damaged DNA. Plasmid R46 not only protected repair-proficient strains but also those with recB, recC, uvrA or lig genotypes, suggesting that R46 protection against bleomycin is independent of these host repair functions. However, R46 protection was abolished in recA or polA strains, indicating that these gene functions are necessary for plasmid-mediated protection. It is suggested that protection may be due to a recA+-dependent interaction of a plasmid-encoded product with host DNA polymerase I, resulting in an increase in the DNA repair capacity of cells. (Author abstract) (47 Refs)

43. A COMPARISON OF THE CYTOGENETIC RESPONSE TO ASBESTOS AND GLASS FIBER IN CHINESE HAMSTER AND HUMAN CELL LINES. DEMONSTRATION OF GROWTH INHIBITION IN PRIMARY HUMAN FIBROBLASTS.

Sincock AM, Delhanty JD, Casey G
Fund for Replacement of Animals in Medical Experiments, London SW20, England
Mutat Res; 101(3):257-268 1982

The response to asbestos and glass fiber was compared for Chinese hamster ovary cells (CHO), primary human fibroblasts (EN1, FPC1, 77LO, 59LO), and human lymphoblastoid cell lines (LIZ and 1526). Dusts employed were crocidolite and chrysotile. CHO cells exposed to the two asbestos fibers showed the expected high level of induced chromosome damage following 48 and 72 hr exposures. A lower level of damage was apparent in cells treated with glass code 100. Coarse glass fibers, code 110, produced no increased chromosome damage. Observed damage due to both types of asbestos and glass 100 consisted mostly of chromosome breaks and rearrangements, and polyploidy after asbestos but not glass. Presence of chrysotile or glass 100 at either 0.05 or 0.1 mg/ml in cultures of adult human fibroblasts resulted in very little increase in cell number over 4 days. Coarse glass code 110 had no effect on growth in fetal or adult human fibroblasts. Lowered cell numbers at harvest in cells treated with chrysotile asbestos of glass code 100 appeared to result from growth inhibition as evidence by lowered mitotic indices in such cultures. A possible reason for the refractoriness of human primary cell lines to cytogenetic damage induced by these agents may be the increased effectiveness of DNA repair in these lines compared to the permanent CHO lines. (19 Refs)

44. MUTAGENIC POTENTIAL OF WATER CONCENTRATES FROM THE EFFLUENT OF A WASTE OIL STORAGE POND.

Brown KW, Donnelly KC
Dept. Soil and Crop Sciences, Texas A and M Univ., College Station, TX, 77843
Bull Environ Contam Toxicol; 28(4):424-429 1982

Water samples collected before and after a waste oil storage pond was dredged contained compounds toxic to Salmonella. Samples collected after dredging exhibited a substantial reduction in the capacity to produce repairable DNA damage in Bacillus subtilis. The reduction in repairable DNA damage may be due to removal of chlorinated hydrocarbons. (14 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

45. CLONING OF TWO GENETICALLY TRANSMITTED MOLONEY LEUKEMIA PROVIRAL GENOMES: CORRELATION BETWEEN BIOLOGICAL ACTIVITY OF THE CLONED DNA AND VIRAL GENOME ACTIVATION IN THE ANIMAL.

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J Virol; 42(3):1088-1098 1982

The Mov-7 and Mov-9 substrains of mice, carrying Moloney murine leukemia virus (M-MuLV) in their germ line at the Mov-7 locus and Mov-9 locus, respectively, are different with respect to virus activation. Infectious virus appears in all mice carrying the Mov-9 locus but is not activated in animals carrying the Mov-7 locus. Consequently, only Mov-9 mice develop viremia and subsequent leukemia. The endogenous M-MuLV provirus with flanking mouse sequences corresponding to the Mov-7 and Mov-9 loci was molecularly cloned. Detailed restriction maps obtained from the cloned DNAs revealed no detectable differences in the proviral genomes. The flanking mouse sequences, however, were different, confirming that the Mov-7 and Mov-9 loci represent different integration sites of M-MuLV. Both clones induced XC plaques in a transfection assay. The specific infectivity of the clones, however, was different. Moreover, NIH 3T3 cells transfected with the Mov-9 clone produced NB-tropic M-MuLV, whereas cells transfected with the Mov-7 clone did not produce infectious virus. The results suggest that M-MuLV integrated at the Mov-7 locus carries a mutation which prevents synthesis of infectious virus but permits XC plaque induction by partial genome expression or synthesis of noninfectious particles. Thus, the pattern of virus expression in Mov-7 and Mov-9 mice correlates with the biological properties of the respective clones. Genomic DNA from Mov-9 mice was not infectious in the transfection assay. As the only difference between the genomic and the cloned Mov-9 DNA appears to be the presence of 5-methylcytosine in CpG sequences, our results suggest that removal of methyl groups by molecular cloning in procaryotes permits genome expression in transfected eucaryotic cells. Our results support the hypothesis that DNA methylation is relevant not only in

genome expression in the animal but also in expression of genes transfected into eukaryotic cells. (28 Refs)

46. INTERACTION OF REACTIVE INTERMEDIATES WITH DNA.

Magee PN, Chu CK, Gombar CT, Jensen DE, Parchman LG
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Adv Exp Med Biol; 136(partB):1335-1347 1982

Covalent binding of some selected chemicals to rat liver DNA is described and the results related to the proposal by Lutz that the extent of binding may be a quantitative indicator in the process of chemical carcinogenesis. Lutz suggested that covalent binding may be expressed in quantitative terms as the Covalent Binding Index relating damage to DNA as a function of dose. N-nitrosodimethylamine was used as a positive control. No in vivo interaction of N-nitrosoproline with rat liver DNA was observed. Since this compound was rapidly eliminated from the body and a very small degree of metabolism occurred, the use of proline as a safe test compound in studies on endogenous nitrosation is supported in animals and humans. A very small, but real interaction between trichloroethylene and mouse liver DNA in vivo was observed, supporting the apparent low carcinogenic potency of the compound and doubts concerning its carcinogenicity. In the presence of sulfhydryl compounds, N-nitrosocimetidine interacted with calf thymus DNA in vitro, yielding a pattern of methylated purine bases identical to that produced by N-methyl-N'-nitro-N-nitrosoguanidine (MNG) and other carcinogenic and mutagenic N-nitroso compounds. Preliminary in vivo experiments indicated that the extent of methylation of DNA was much less in organs of animals treated with nitrosocimetidine than those treated with MNG. (37 Refs)

47. CHEMICAL CARCINOGENESIS: INTERACTIONS OF CARCINOGENS WITH NUCLEIC ACIDS.

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Dept. Pathology, Univ. Toronto, Toronto, Ontario, Canada
Cancer - A Comprehensive Treatise Vol. 1. Etiology - Chemical and Physical Carcinogenesis. Second Edition. Becker FF, ed. New York, Plenum Press, 714 pp., 1982.

The nature of modified nucleic acids following interactions with carcinogens is reviewed. Interactions of chemical carcinogens with DNA include covalent interactions such as alkylating agents, nitrosamines and nitrosamides, and noncovalent interactions of both internal and external binding types. Purine-N-oxides and many inorganic metal compounds are known to be carcinogenic. The use of fractionation techniques has revealed that carcinogens exhibit a preferential interaction with some regions of DNA in chromatin. Interactions of chemical carcinogens with RNA have been studied, and alterations in RNA have been attributed to alkylating agents such as nitrosamines and nitrosamides, to urethan, to aromatic amines and amides, and to polycyclic aromatic hydrocarbons. Interactions of carcinogens with nucleic acids exert a profound effect on the structure and synthesis of DNA and RNA, gene expression, and regulation of cellular development. Although the exact mechanism or mechanisms by which DNA damage and repair are involved in carcinogenesis is not completely understood, there is overwhelming evidence indicating a causal relationship between these two processes. (634 Refs)

48. CONSTITUTIVE AND CARCINOGEN-DERIVED DNA BINDING AS A BASIS FOR THE ASSESSMENT OF POTENCY OF CHEMICAL CARCINOGENS.

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Adv Exp Med Biol; 136(partB):1349-1365 1982

The hypothesis that ground-level DNA damage is unavoidable and must be regarded as constitutive to a cell is presented. Sources for this type of damage include physiological reactive intermediates, nitrosamines, irradiation, carcinogenic metals, and viruses. Carcinogens are classified as initiating (DNA damaging, genotoxic) and nongenotoxic (act by modulating reactions and side reactions that increase or constitutive DNA damage or enhance the chance for such damage to proceed to a tumor). Carcinogenic potency of a carcinogen is defined as a product of persistent DNA damage x mutagenicity x nongenotoxic modulation of DNA damage. A correlation of DNA binding in vivo and the form of a 'Covalent Binding Index' (CBI) to the carcinogenic potency is shown. The CBI equals (micromole chemical bound/mole DNA nucleotides)/(millimole chemical administered/kg animal wt). Good linear correlation was found with an approx uncertainty of the estimate of a factor of 10 with a total span of values of 10(6). DNA binding is not enough to describe the potency of carcinogens; additional information is necessary to assess the organ specificity of initiating carcinogens. When administered alone, the nongenotoxic carcinogens appear to be much less potent than initiating carcinogens. Nongenotoxic carcinogens cannot be detected on the basis of chemical structure and short-term tests of their carcinogenic potency are not available. (21 Refs)

49. FORCE FIELD CONFORMATIONAL ANALYSIS OF AMINOFLUORENE AND ACETYLAMINOFLUORENE SUBSTITUTED DEOXYGUANOSINE.

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Chem Biol Interact; 40(1):57-76 1982

The hepatocarcinogen N-hydroxy-2-acetylaminofluorene forms two C8-substituted deoxyguanosine adducts in vivo. The conformation of these adducts, as well as 2'-deoxyguanosine and 8-amino-2'-deoxyguanosine has been studied with Allinger's force field. Using the glycoside rotation as a reaction coordinate, multidimensional potential energy surfaces were determined by relaxing all internal degrees of freedom. The calculations indicate the 2'-deoxyguanosine should exist as a mixture of syn and anti forms, that the syn form is slightly favored for 8-amino-2'-deoxyguanosine, that N-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-C8-AAF) will only be found in the syn conformation and that, although the syn form is also more stable for N-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF), it will have a substantially greater proportion of the anti-conformer than is found with dG-C8-AAF. The results of the force field calculations are discussed in relation to the effects these adducts may have on DNA structure. (Author abstract) (55 Refs)

50. MORPHOLOGICAL TRANSFORMATION, DNA STRAND SEPARATION, AND ANTINUCLEOSIDE IMMUNOREACTIVITY FOLLOWING EXPOSURE OF CELLS TO INTERCALATING DRUGS.

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Mol Pharmacol; 21(3):739-743 1982

Two structurally similar alkylating agents, the acridine derivatives proflavine and quinacrine were compared for their ability to induce morphological transformation and DNA strand separation in mouse 3T3 cells. The technique of antinucleoside immunofluorescence staining was used to detect the unwinding of DNA in nuclei of fixed cells. Treatment with proflavine resulted in higher transformation frequencies than were found with quinacrine. Results suggest that DNA strand separation is, or at least accompanies, an early step in cell transformation by intercalating drugs. (28 Refs)

51. CORRELATION OF NITROSOUREA MURINE BONE MARROW TOXICITY WITH DEOXYRIBONUCLEIC ACID ALKYLATION AND CHROMATIN BINDING SITES.

Green D, Tew KD, Hisamatsu T, Schein PS
Div. Medical Oncology, Georgetown Univ. Hosp.-2230, 3800 Reservoir Road, N.W., Washington, DC, 20007
Biochem Pharmacol; 31(9):1671-1679 1982

All of the clinically available nitrosourea antitumor agents produce serious treatment-limiting bone marrow toxicity. A reduction in this toxicity can be achieved by attaching the chloroethylnitrosourea cytotoxic group to C2 (chlorozotocin) or C1 (1-(2-chloroethyl)-3-(beta-D-glucopyranosyl)-1 nitrosourea, GANU) of glucose. Both glucose analogs are less myelotoxic in mice than 1-(2-chloroethyl)-3-cyclohepyl-1-nitrosourea (CCNU) or 1-(4-amino-2-methylpyrimidin-5-yl)methyl-3-(2 chloroethyl)-3-nitrosourea (ACNU), while retaining comparable antitumor activity against the murine L1210 leukemia. To define the nuclear mechanisms for this reduced myelotoxicity, alkylation of L1210 and murine bone marrow DNA was quantitated. With the use of the endonucleases micrococcal nuclease and DNase I, the sites of alkylation within the chromatin substructure were determined. Experiments were performed on L1210 leukemia or bone marrow cells that had been incubated in vitro for 2 hr with 0.1 mM [14C]chloroethyl drug. The quantitative alkylation of DNA by GANU was 1.3-fold greater in L1210, as compared to bone marrow, cells. This ratio of DNA alkylation is comparable to the 1.3 ratio we previously reported for chlorozotocin. In contrast, the ratio of alkylation (L1210:bone marrow DNA) for the myelotoxic ACNU was 0.66, similar to 0.59 for CCNU. Nuclease digestion experiments demonstrated that chlorozotocin and GANU preferentially alkylated internucleosomal linker regions of bone marrow chromatin, while nucleosome core particles were the preferred targets of CCNU and ACNU. The reduced myelotoxicity of chlorozotocin and GANU may be correlated with the advantageous ratio of L1210: bone marrow DNA alkylation and preferential alkylation of internucleosomal regions of bone marrow chromatin. (Author abstract) (35 Refs)

52. CONFORMATION CHANGE OF DNA INDUCED BY 6-OXYBENZO[A]PYRENE RADICAL.

Sato Y, Okano T
Pharmaceutical Inst., Tohoku Univ., Aobayama, Sendai 980, Japan
Gan; 73(2):308-314 1982

The interaction of 6-oxybenzo[a]pyrene radical with DNA with 50% methanol solution was studied in order to determine whether the radical molecule influences DNA conformation. The mode of the interaction of 6-oxybenzo[a]pyrene radical with DNA differed from that of other benzo[a]pyrene derivatives such as 6-hydroxybenzo[a]pyrene, diol-epoxides, etc. The physical binding of the radical molecule to DNA seemed to be transient. This physical binding is most likely due to adsorption at the external surface of DNA, ie, simply contact in character. It is likely that the local base-pairs are tilted by the contact due to hydrophobic interactions. Furthermore, it is concluded that the covalently bound radical molecule, which is mainly introduced on guanine base, induces a twist of the base plane with respect to other base planes. (Author abstract) (34 Refs)

53. VP16-213 AND PODOPHYLLOTOXIN: A STUDY ON THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY.

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Cancer Chemother Pharmacol; 7(2/3):103-111 1982

The chemical structure of VP16-213 (4'-demethyl epipodophyllotoxin ethylidene beta-D glucoside) differs from podophyllotoxin (PPT) at three positions, resulting in different biological properties with respect to microtubule assembly in vitro, intracellular degradation of DNA in HeLa cells, and nucleoside transport in HeLa cells. PPT inhibits microtubule assembly in vitro, while VP16-213 has no effect. Previous cell cycle studies showed that both drugs are cytotoxic in either the late S or early G2 phase. In VP16-213-treated HeLa cells, high mol wt DNA was converted to lower mol wt forms; the effect was dose and temperature dependent and reversible after drug washout. Conversely, PPT did not cause DNA breakage, probably due to structural differences with V which contains a 4' hydroxyl group lacking in the former. Both drugs inhibit the saturable rate component of nucleoside transport into HeLa cells and do not inhibit nucleoside phosphorylation. VP16-213 has been found to be effective in chemotherapy of leukemia and solid tumors. The use of VP16-213 in combination with other cytotoxic nucleoside analogs is discussed. (52 Refs)

54. STRUCTURE-ACTIVITY RELATIONSHIPS INVOLVED IN THE SITE-SPECIFIC FRAGMENTATION OF LINEAR DUPLEX DNAS BY TALISOMYCIN AND BLEOMYCIN ANALOGS.

Mirabelli CK, Huang CH, Prestayko AW, Crooke ST
Dept. Pharmacology, Baylor Coll. Medicine, Texas Medical Center, Houston, TX, 77030
Cancer Chemother Pharmacol; 8(1):57-65 1982

Site-specificity of DNA breakage by bleomycin (BLM) and talisomycin (TLM) was examined using analogs of the parent compounds and two species of DNA. Fragmentation of Hind III- and PstI-digested PM2 DNA and Hind III-digested pBR322 DNA by BLM A2 and B2 and TLM A, B, S2b, and S10b was compared. With PM2 DNA as a substrate, TLM A was more active than BLM A2 in inducing double strand breakage of whole linear PM2 DNA. Ethidium bromide staining band patterns produced from inactivation of endonuclease-digested DNA with BLM or TLM differed, indicating that certain PM2 genome segments serve as better substrates for double strand fragmentation by BLM or TLM. (31 Refs)

55. DIRECT EFFECT OF DIAGNOSTIC ULTRASOUND ON GENETICALLY INTERESTING MOLECULES: A THEORETICAL MODEL.

Ciatti S, Domokos G, Kovesi-Domokos S, Milano F
Cattedra di Radiologia 'R', Universita degli Studi di Firenze, Firenze, Italy
Br J Cancer; 45(Suppl5):!99-201 1982

Three mechanisms responsible for ultrasound-induced changes in DNA are examined: structural changes in the molecule due to sound absorption from a high harmonic of the repetition frequency of a pulsed ultrasound radiation, structural changes due to multiphonon absorption from low harmonics of the repetition frequency, and break-up of the molecule as a consequence of excitation of collective vibrations. (10 Refs)

56. THE ACTIVATING ROLE OF GLUTATHIONE IN THE MUTAGENICITY OF 1,2-DIBROMOETHANE.

van Bladeren PJ, Breimer DD, Rotteveel-Smijs GM, Hoogeterp JJ, Mohn GR, De Groot A, Van Zeeland AA, Van der Gen A
Dept. Pharmacology, Univ. Leiden, P.O. Box 9502, 2300 RA Leiden, The Netherlands
Adv Exp Med Biol; 136(partA):809-820 1982

Role of conjugation to glutathione (GSH) in the mutagenicity of 1,2-dibromoethane (DBE) was investigated and some preliminary results on the occurrence of reactive GSH conjugates in vivo are reported. The activity of GSH-S-transferase in vitro, synthesis and reactions of model conjugates, mutagenicity of DBE, and DNA binding of DBE are reviewed. DBE was tested to determine which mechanism is responsible for the activation. Results indicated that the enhancement of mutagenic activity is caused by conjugation to GSH. The spontaneous binding of DBE to DNA may result from initial reactions with sulfur or nitrogen nucleophiles, giving rise to the same type of half-mustards as the GSH conjugates. Rats excreted 30-50% of DBE in the form of mercapturic acid. Direct conjugation of GSH with DBE and DBE oxidation followed by loss of hydrogen bromide may lead to the structure of the metabolite. Excretion of N-acetyl-S-2-hydroxyethyl-L-cysteine by male Wistar rats after administration of 0.1 millimole DBE/rat with and without pretreatment with 10 g 1-phenylimidazole/rat is presented. Results show that direct conjugation of DBE to GSH and thus formation of the reactive 2-halogenothioether in vivo is a viable pathway of metabolization. Conjugation to GSH can give rise to the formation of reactive glutathione conjugates, provided a second leaving group is present at the carbon atom next to the one where sulfur substituent becomes attached. (21 Refs)

57. THE INDUCTION OF SCE AND CHROMOSOMAL ABERRATIONS WITH RELATION TO SPECIFIC BASE METHYLATION OF DNA IN CHINESE HAMSTER CELLS BY N-METHYL-N-NITROSOUREA AND DIMETHYL SULPHATE.

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Carcinogenesis; 3(4):385-390 1982

Chinese hamster cells (V79) were treated, either as exponentially proliferating cultures or under conditions where they were density-inhibited, with various doses of the potent carcinogen N-methyl-N-nitrosourea (MNU) or the relatively weak carcinogen dimethylsulfate (DMS). The colony forming ability of these cells and the induced frequencies of sister chromatid exchanges (SCEs) and chromosomal aberrations were assayed. Following the exposure of density-inhibited cells to radio-labeled methylating agents (labeled in the methyl group) these phenomena were related to the levels of 7-methylguanine (7-meGua), O6-methylguanine (O6-meGua) and 3-methyladenine (3-meAde) in the DNA. At equitoxic doses MNU and DMS induced similar frequencies of SCEs and chromosomal aberrations. Since, at equitoxic doses, MNU produces approx 20x more O6-meGua in V79 cell DNA than does DMS, this indicates that the formation of O6-meGua in DNA is not a major cause of SCEs and chromosomal aberrations. DMS-induced SCEs may be mediated via the production of both 3-meAde and 7-meGua in the DNA; these two methylated purines may also be responsible for MNU-induced SCEs. Therefore, no one specific methylated purine was identified as being solely accountable for the formation of SCEs. Also, the repair of lesions in the DNA of non-replication V79 cells leads to a reduction in the SCE frequency on their subsequent release from the density-inhibited state, suggesting that repair is not intimately responsible for their formation. No association was discernable between chromosomal aberrations and any of the three methylated purines studied. (Author abstract) (34 Refs)

58. DNA METHYLATION IN MOUSE CELLS IN CULTURE AS MEASURED BY RESTRICTION ENZYMES.

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Biochim Biophys Acta; 697(1):53-59 1982

Methylation of DNA in normal mouse cultured 3T3 cells and in their virally or chemically transformed derivatives was studied. DNA methylation was studied by restriction with HpaII, MspI, or HpaII plus MspI. DNA from the chemically transformed cells was cleaved about twice as often with HpaII than was the DNA of normal and virally transformed cells. Digests with MspI and HpaII plus MspI were identical in all cell lines studied. Densitometry of the restriction patterns allowed an estimate of total DNA methylation from the wt av lengths. The chemically transformed cell line showed 25% reduction in methylation compared to the other cell lines. Southern blot hybridization using satellite DNA showed that these sequences followed a pattern of modification similar to that of total DNA. (Author abstract) (47 Refs)

59. EXPRESSION AND METHYLATION OF THE MOUSE ALPHA-FETOPROTEIN GENE IN EMBRYONIC, ADULT, AND NEOPLASTIC TISSUES.

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J Biol Chem; 257(9):5148-5153 1982

Expression of the mouse alpha-fetoprotein gene in embryonic, adult, and neoplastic tissues was assessed by RNA dot hybridization using 32P-labeled alpha-fetoprotein cDNA as probe. Alpha-fetoprotein mRNA was present in high levels in total RNA from yolk sac endoderm, fetal liver, and an alpha-fetoprotein-producing hepatoma. In contrast, this mRNA was greatly depleted in total RNA from yolk sac mesoderm and essentially absent in brain, adult liver, and a non-alpha-fetoprotein-producing hepatoma. These results indicated that alpha-fetoprotein gene expression was controlled primarily at the transcriptional level. The presence of the modified base, 5-methylcytosine, in the alpha-fetoprotein gene was studied by comparing hybridization patterns obtained by Southern blot analysis of DNA cleaved with the restriction isoschizomers endonuclease

Msp I and endonuclease Hpa II. The gross sequence organization and reiteration frequency of the alpha-fetoprotein gene were invariant among the DNA samples, whereas, in each case, there was a positive correlation between hypomethylation of six CCGG (Hpa II) sites in the alpha-fetoprotein gene and expression of this gene. These Hpa II sites were distributed throughout a large portion of the alpha-fetoprotein gene. Patterns of cytosine methylation in this gene were established before day 15 of gestation in yolk sac endoderm and mesoderm. (Author abstract) (46 Refs)

60. AMOUNT AND DISTRIBUTION OF 5-METHYLCYTOSINE IN HUMAN DNA FROM DIFFERENT TYPES OF TISSUES OR CELLS.

Ehrlich M, Gamma-Sosa MA, Huang LH, Midgett RM, Kuo KC, McCune RA, Gehrke C
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Nucleic Acids Res; 10(8):2709-2721 1982

Analysis of the total base composition of DNA from seven different normal human tissues and eight different types of homogeneous human cell populations revealed considerable tissue-specific and cell-specific differences in the extent of methylation of cytosine residues. The two most highly methylated DNAs were from thymus and brain with 1.00 and 0.98 mole percent 5-methylcytosine (m5C), respectively. The two least methylated DNAs from in vivo sources were placental DNA and sperm DNA, which had 0.76 and 0.84 mole percent m5C, respectively. The differences between these two groups of samples were significant with p less than 0.01. The m5C content of DNA from six human cell lines or strains ranged from 0.57 to 0.85 mole percent. The major and minor base composition of DNA fractionated by reassociation kinetics was also determined. The distribution of m5C among these fractions showed little or no variation with tissue or cell type with the possible exception of sperm DNA. In each case, nonrepetitive DNA sequences were hypomethylated compared to unfractionated DNA. (Author abstract) (44 Refs)

61. ON THE CHROMATIN STRUCTURE OF THE AMPLIFIED, TRANSCRIPTIONALLY ACTIVE GENE FOR DIHYDROFOLATE REDUCTASE IN MOUSE CELLS.

Barsoum J, Levinger L, Varshavsky A
Dept. Biology, Massachusetts Inst. Technology, Cambridge, MA, 02139
J Biol Chem; 257(9):5274-5282 1982

The method for two-dimensional hybridization mapping of nucleosomes and was used to analyze chromatin structure of the gene for dihydrofolate reductase (DHF reductase; 5,6,7,8-tetrahydrofolate:NADP+ oxidoreductase) in L5178Y-R mouse cells. The DHF reductase gene in these cells is amplified about 350-fold as a result of selection for resistance to methotrexate. Dramatic overproduction of DHF reductase mRNA in L5178Y-R cells suggests that most of the DHF reductase genes in these cells are transcribed. We report that all major mononucleosomal species resolvable by two-dimensional fractionation are detected by both DHF reductase- and satellite DNA-specific hybridization probes. Although the DHF reductase and satellite hybridization patterns differ somewhat from each other and from the total mononucleosomal pattern, their overall similarity is very high. In particular, no large differences in the abundance of mononucleosomes containing high mobility group non-histone proteins (HMG) 14 and 17 are seen between the DHF reductase and satellite chromatin regions under a wide variety of conditions for chromatin isolation, digestion, and fractionation. Possible interpretations of the apparent lack of selectivity of HMG-chromatin interactions in this system are discussed. We also found that the amplified DHF reductase genes possess a wide range of nucleosomal repeat lengths close to that in the bulk chromatin. In contrast, the range of nucleosomal repeat lengths in the satellite chromatin is much narrower than in both DHF reductase and bulk chromatin. (Author abstract) (56 Refs)

62. GAMMA HEAVY CHAIN DISEASE IN MAN: CDNA SEQUENCE SUPPORTS PARTIAL GENE DELETION MODEL.

Alexander A, Steinmetz M, Barritault D, Frangione B, Franklin EC, Hood L, Buxbaum JN
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Proc Natl Acad Sci USA; 79(10,part1):3260-3264 1982

Human gamma heavy chain disease (HCD) is characterized by the presence in serum of a short monoclonal Ig gamma chain unattached to light chains. Although most HCD proteins have internal deletions, in some the defect is NH2-terminal. The OMM gamma3 HCD serum protein is of the latter type, having undergone an extensive NH2-terminal deletion with a sequence starting within the hinge. A cell line synthesizing the OMM protein has enabled us to study the biogenesis of the abnormal molecule. In vitro translation of isolated mRNA yields a protein containing a hydrophobic NH2-terminal leader sequence. In the intact cell, the precursor molecule is processed normally to yield a protein with an NH2-terminal sequence homologous to the beginning of the variable (V) region. The nucleotide sequence of cDNA prepared from the OMM mRNA encodes a 19-amino acid leader followed by the first 15 residues of the V region. An extensive internal deletion encompasses the remainder of the V and the entire CH1 domain. Immediately following the short V region, there is information in the cDNA for the entire normal hinge. The primary synthetic product is thus an internally deleted molecule that undergoes postsynthetic degradation to yield the NH2-terminally deleted serum protein. The structure of the OMM mRNA suggests that the protein abnormality results from a partial gene deletion rather than defective splicing. (Author abstract) (45 Refs)

63. THE SMALL CHROMATIN FRAGMENTS RELEASED BY MICROCOCCAL NUCLEASE FROM HEPATOMA TISSUE CULTURED CELL NUCLEI ARE STRONGLY ENRICHED IN CODING DNA SEQUENCES AND ARE RELATED TO AN ACTIVELY TRANSCRIBED SINGLE-STRANDED DNA FRACTION.

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Biochim Biophys Acta; 697(1):60-70 1982

It was shown with the use of specific probes that mild micrococcal nuclease digestion releases from chromatin actively-transcribed genes as small nucleosome oligomers. In the present work we demonstrate that most if not all of the active genes are accessible to the nuclease. It was found that the short released fragments are greatly enriched in transcribed DNA sequences, the most enriched being the dimers of nucleosomes since 35% of their DNA could be hybridized to cytoplasmic RNA. The results of cDNA-DNA hybridizations indicate that the monomers and dimers of nucleosomes contain most of the DNA sequences which encode poly(A+) RNAs, however larger released fragments include some transcribed sequences,

while the nuclease-resistant chromatin is considerably impoverished in coding sites. These evidences and the finding that about 25% of the DNA from the dimers of nucleosomes are exclusively located in this class of fragments, tend to prove that the active chromatin regions are attacked in a non-random way by micrococcal nuclease. We have previously isolated, without using exogenous nuclease, an actively transcribed genomic fraction amounting to 1.5-2% of the total nuclear DNA, formed of single-stranded DNA. In the present study we show that all or nearly all the single-stranded DNA sequences could be reassociated with the DNA fragments present in the released monomers and dimers of nucleosomes. Our observations confirmed our previous finding that the greatest part of single-stranded DNA selectively originates from the coding strand of genomic DNA. (Author abstract) (34 Refs)

64. IMMUNOGLOBULIN HEAVY CHAIN GENE REARRANGEMENT AND TRANSCRIPTION IN MURINE T CELL HYBRIDS AND T LYMPHOMAS.

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Proc Natl Acad Sci USA; 79(9):3015-3019 1982

We have examined the arrangement of immunoglobulin heavy chain constant (CH) and joining (JH) region genes in murine T cell hybrid lines and in T lymphomas. CH genes derived from both parental cell types were present in all hybrids for which polymorphism in sequences flanking CH genes permitted us to distinguish parental CH genes. All T lymphomas and T cell hybrids retained the Calpha gene in germ-line configuration and all but one cell line had germ-line Cmu genes. Novel DNA fragments reactive with JH probes were observed in six of nine T cell hybrids, as well as in two T lymphomas, WEH17.1 and YAC-1, but not in the fusion parent, BW5147. No RNA homologous to Cgamma2b, Calpha, or lambda genes was detected in any of the T cell lines. T cell lines contained poly(A)+ RNA homologous to a Cmu cDNA probe. More importantly, in several cell lines the Cmu RNAs were associated with membrane-bound polyribosomes. These results suggest that both JH rearrangements and Cmu RNA production occur in at least some mature, antigen-specific T cells. They may therefore reflect events in normal T cell development and function related to those involved in the generation of the T receptor for antigen. (Author abstract) (30 Refs)

65. SIMULTANEOUS EXPRESSION OF IMMUNOGLOBULIN MU AND DELTA HEAVY CHAINS BY A CLONED B-CELL LYMPHOMA: A SINGLE COPY OF THE VH GENE IS SHARED BY TWO ADJACENT CH GENES.

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Proc Natl Acad Sci USA; 79(9):2996-3000 1982

The cloned murine B-cell lymphoma line (BCL1) that expresses surface IgM and IgD is considered to be a model for the immunoglobulin gene expression of the mature virgin B cell. Of particular interest is the mechanism by which a single VH gene is shared by two CH genes. We examined the organization of the immunoglobulin heavy chain genes in BCL1 DNA. A single arrangement of CH genes was found with the expressed VHDJH gene complex just 5' to the C mu gene. The complete DNA sequence of the VH gene was determined. No rearrangement occurred in the intervening DNA between the JH and C mu genes or between the C mu and C delta genes. We conclude that dual expression of mu and delta heavy chains using a single VH gene is accomplished by alternate processing of a primary transcript that encompasses the VHDJH complex and both CH genes. (Author abstract) (36 Refs)

66. MOLECULAR CLONING OF HUMAN IMMUNE INTERFERON CDNA AND ITS EXPRESSION IN EUKARYOTIC CELLS.

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Nucleic Acids Res; 10(8):2487-2501 1982

Starting with mRNA derived from Staphylococcal enterotoxin A induced human splenocytes, dsDNA was synthesized and inserted into the unique BamHI site of the eukaryotic expression vector pSV529. A recombinant plasmid containing human immune interferon (IFN-gamma) cDNA was identified by hybridization of plasmid inserted DNA bound onto nitrocellulose filters with mRNA derived from SEA-induced splenocytes, translation of the eluted RNA in Xenopus laevis oocytes and assaying from IFN activity. Plasmids containing the entire human IFN-gamma cDNA sequence were identified by colony hybridization and were sequenced. A unique coding region was identified which predicted a protein of 166 amino acids, the 20 N-terminal amino acids of which presumably represent a signal peptide. After transfection of monkey cells with plasmid DNA isolated from one of the recombinant clones (pHIIF-SV-gamma1), IFN was excreted into the culture medium. This IFN was not distinguishable from human IFN-gamma by serological criteria or by cell target species specificity. (Author abstract) (53 Refs)

67. EXPRESSION OF THE GENE ENCODING THE ADENOVIRUS DNA TERMINAL PROTEIN PRECURSOR IN PRODUCTIVELY INFECTED AND TRANSFORMED CELLS.

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J Virol; 42(2):488-501 1982

The major product of in vitro translation of early RNA prepared from H5ts125-infected cells and selected by hybridization to adenoviral DNA fragments spanning the region from 14.7 to 31.5 map units has been shown to be identical to the 87-kilodalton terminal protein precursor. A 72-to 75-kilodalton polypeptide whose mRNA can be selected by DNA from this region and made in the presence of anisomycin was indistinguishable from the 72-kilodalton single-stranded DNA-binding protein encoded by the region from 60.1 to 66.6 map units. The accumulation of cytoplasmic RNA sequences complementary to these l-strand genes under various conditions of infection and in certain lines of transformed cells has been investigated by solution hybridization of cytoplasmic RNA to the separated strands of restriction endonuclease fragments of adenoviral DNA. During the early phase, RNA sequences complementary to the region from 11.6 to 36.7 map units were present at a concentration of 10 to 60 copies per cell, regardless of the nature of the block used to inhibit viral DNA synthesis. By 24 hr after infection in the absence of any such block, sequences complementary to the regions from 11.6 to 18.2 map units (IVa2) and from 18.6 to 36.7 map units (E2B) accumulated to concentrations of 4,800 and 280 copies per cell,

respectively. The ratio of cytoplasmic E2A RNA sequences to E2B RNA sequences remained close to 10:1 throughout the time period investigated. Of the transformed cell lines which retained E2B DNA sequences that were examined, only the T2C4 line expressed these sequences in cytoplasmic RNA. The implications of these observations for regulation of expression of the adenoviral early l-strand genes are discussed. (Author abstract) (73 Refs)

68. DIFFERENT FORMS OF SIMIAN VIRUS 40 LARGE TUMOR ANTIGEN VARYING IN THEIR AFFINITIES FOR DNA.

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J Virol; 42(2):456-466 1982

In various permissive monkey cell lines infected with simian virus 40 there are two major forms of large T antigen which differ in their rate of sedimentation through sucrose gradients. The lighter (5 to 7S) form sedimented slightly more rapidly than the 4S tRNA marker, whereas the heavier (16S) form sedimented slightly more slowly than the 18S rRNA marker. The small t antigen did not form complexes which sedimented as rapidly as those formed by the large T antigen. The 16S T antigen form was converted to the slowly sedimenting 5 to 7S form in the presence of 1.0 M NaCl. The majority of large T antigen synthesized in cell-free protein-synthesizing systems primed by mRNA isolated from infected cells sedimented as the 5 to 7S form even when premixed with excess quantities of cellular T antigen. The formation of the 16S form in infected cells did not require ongoing viral or cellular DNA replication because considerable quantities of this T antigen class were produced in the presence of DNA synthesis inhibitors, such as cytosine cytosine arabinoside. Both 5 to 7S and 16S forms could be isolated separately and, therefore, each could be analyzed as to its individual properties. The 5 to 7S T antigen form bound more efficiently and tightly to DNA and had specific affinity for sequences at the viral origin of replication, whereas the 16S form bound less efficiently to DNA and exhibited very little specificity for origin-containing DNA sequences. It is therefore likely that the active DNA-binding species of T antigen isolated from infected cells is the 5 to 7S form. (Author abstract) (43 Refs)

69. MUTANT OF SIMIAN VIRUS 40 LARGE T-ANTIGEN THAT IS DEFECTIVE FOR VIRAL DNA SYNTHESIS, BUT COMPETENT FOR TRANSFORMATION OF CULTURED RAT CELLS.

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J Virol; 42(3):854-864 1982

A mutant was isolated which demonstrates that the transforming activity of simian virus 40 large T-antigen is separable from its function in viral DNA replication. The mutant, SVR9D, is nonconditionally defective for viral DNA synthesis, but competent at wild-type level for morphological transformation of cultured rat cells. The lytic growth defect in SVR9D is complemented by the simian virus 40 A gene product present in the transformed CV1 cell line, COS1. The lesion in SVR9D DNA was mapped genetically by marker rescue of plaque formation and localized to a 214-base-pair segment of the viral genome bounded by nucleotide numbers 4100 and 4314. DNA sequence analysis showed the mutation to be an adenine-to-guanine transition at nucleotide number 4178. This change predicts a lysine-to-glutamic acid amino acid change at residue number 214 of the mutant large T-antigen polypeptide. (Author abstract) (32 Refs)

70. DIFFERING POPULATIONS OF INRACISTERNAL A-PARTICLE GENES IN MYELOMA TUMORS AND MOUSE SUBSPECIES.

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J Virol; 42(2):411-421 1982

Intracisternal A-particle genes form a family of endogenous retrovirus-like genetic elements that are transcribed in mouse plasmacytomas (myeloma tumors). Two types of A-particle genes that can be differentiated by a sequence of 0.5 kilobase found in one type but not the other have been identified. Quantitative Southern blot analysis was used to measure the populations of different A-particle genes in DNAs from BALB/c mice, the Japanese subspecies Mus musculus subsp molossinus, and myeloma tumors. The majority of the genes (715 copies per haploid genome or 76%) were found to be nearly identical except for small changes in conserved restriction enzyme sites. The second type of A-particle gene was much less abundant with 90 copies representing approx 10%. The A-particle RNA in MOPC104E and MOPC315 was found to be colinear with a small portion of this latter type, comprising only 2% of the endogenous intracisternal A-particle sequences. Myeloma tumor DNA was found to have a two- to fourfold increase in the number of these genes, suggesting that the intracellular viruses have been activated to produce a double-stranded complementary DNA which subsequently integrated into the tumor genome. Analysis of M musculus subsp molossinus DNA revealed similar but shifted populations of A-particle genes, when compared with BALB/c DNA, except for the absence of a prominent EcoRI-HindIII band at 3.9 kilobases. This latter band, representing approx 15% of the A-particle genes in BALB/c DNA, was shown to be a deletion variant of the most abundant gene family. (Author abstract) (37 Refs)

71. ISOLATION AND CHARACTERIZATION OF C-MYC, A CELLULAR HOMOLOG OF THE ONCOGENE (V-MYC) OF AVIAN MYELOCYTOMATOSIS VIRUS STRAIN 29.

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J Virol; 42(3):773-779 1982

The chicken genome contains nucleotide sequences homologous to the transforming genes (oncogenes) of a number of avian retroviruses. We have isolated chicken DNA (c-myc) that is homologous to the oncogene (v-myc) of the avian myelocytomatosis virus MC29 and have compared the structures of the cellular and viral genes. Results from restriction endonuclease mapping of c-myc and from analysis of heteroduplexes between the DNAs of the cellular and viral genes show that c-myc is homologous to 1,500 nucleotides in v-myc DNA. This homologous region is interrupted in c-myc by an intron-like sequence of 1,100 nucleotides which is absent from v-myc. Nuclear RNA from normal chicken cells contains at least five species of transcripts from c-myc ranging from 2.5 to 6.5 kilobases in length. By contrast, cytoplasm contains only the 2.5-kilobase c-myc RNA. These features of the c-myc gene and its nuclear transcripts are characteristic of normal cellular genes and suggest that the myc gene is of cellular rather than viral origin. The exons in c-myc may define two functional domains in the gene and may therefore facilitate the dissection of the different

oncogenic potentials of the MC29 virus. (Author abstract) (28 Refs)

72. ISOLATION OF A CDNA CLONE FOR THE MURINE TRANSPLANTATION ANTIGEN H-2KB.

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Proc Natl Acad Sci USA; 79(10,part1):3270-3274 1982

A library of cloned cDNAs constructed from the poly(A)+RNA of the murine thymoma cell line EL4 (b haplotype) was screened with a probe encoding a short region of the H-2Kb transplantation antigen. One of the clones isolated, pH202, contains a region that can code for a transplantation antigen with an amino acid sequence 98% homologous to that previously published for H-2Kb. Based on this high degree of homology, pH202 appears to encode the H-2Kb antigen from amino acid 66 through the carboxy terminus, including 386 nucleotides of 3'-untranslated sequence. The amino acid sequence deduced from pH202 suggests that the H-2Kb antigen is actually two amino acids longer than previously reported (a total of 348 residues). Four other differences in amino acid assignments are seen. Analysis of the DNA sequences of pH202 and other H-2 clones previously described in the literature suggests that alternative routes of splicing at the 3' end of the coding region are involved in the production of different transplantation antigen mRNAs. (Author abstract) (27 Refs)

73. DEPENDENCE OF TUMOR-FORMING CAPACITIES OF CELLS TRANSFORMED BY RECOMBINANTS BETWEEN ADENOVIRUS TYPES 5 AND 12 ON EXPRESSION OF EARLY REGION 1.

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J Virol; 42(2):708-718 1982

Recombinants between an adenovirus Type 5 (Ad5) deletion mutant and the Ad12 DNA fragment containing early Region 1 (E1) were isolated from cells cotransfected with the EcoRI-C fragments of Ad12DNA and Ad5 dl312 (deletion in E1A) DNA (rcA) and from cells cotransfected with the SalI-C fragment of Ad12 DNA and Ad5 dl312 DNA (rcB). No recombinant was isolated from cells cotransfected with Ad5 dl313 (deletion in E1B) DNA and restriction fragments of Ad12 DNA. Both rcA and rcB are defective and able to replicate in human embryo kidney (HEK) and KB cells with complementation by dl312. Both rcA and rcB formed Ad12 T antigen g, but not T antigen f, in infected HEK and KB cells. In rcA- and rcB-infected cells, Ad5 E1B and Ad12 E1A genes are transcribed. Heteroduplex and size analysis of rcA-1 or rcB-1 DNA fragments hybridized with Ad12 DNA revealed that rcA-1 DNA has a deletion between 5 and 15 map units with an insertion of a portion of Ad12 DNA (10%) and that rcB-1 DNA has a deletion between 70 and 80 map units with an insertion of a portion of Ad12 DNA (10%). The transformed cell lines, RCAY and RCBY, were established after infection of rat 3Y1 cells with rcA and rcB, respectively. Both Ad5 and Ad12 DNA sequences are contained in these cells. In RCAY cells, Ad12 T antigen g is detected, but Ad12 T antigen f is not. In RCBY cells, both Ad12 T antigens g and f are detected. Only the Ad12 E1A gene is transcribed in RCAY cells, whereas Ad5 E1B, Ad12 E1A, and Ad12 E1B genes are transcribed in RCBY cells. In soft-agar cultures, RCBY cells form large colonies, whereas RCAY cells form only tiny colonies. RCBY cells form tumors as efficiently as 12WY cells in transplanted rats. RCAY cells formed tumors inefficiently. Ad5-transformed 5WY cells do not form tumors. These observations indicate that the efficient tumor formation by RCBY cells is dependent on the expression of the Ad12 E1A and E1B genes, whereas the inefficient tumor formation by RCAY cells is due to the expression of only the Ad12 E1A gene. (Author abstract) (28 Refs)

74. ENCAPSIDATION AND EXPRESSION OF THE HERPES THYMIDINE KINASE GENE IN POLYOMA VIRUS.

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J Virol; 42(2):691-699 1982

A recombinant DNA of 5,150 base pairs was prepared containing the intact early region of polyoma virus, including the viral origin of replication and the structural sequences of the herpes simplex virus Type 1 thymidine kinase gene. Although no thymidine kinase activity was detected when herpes structural sequences alone were transfected into cells, activity was produced when the structural gene followed the polyoma early region. The recombinant DNA was encapsidated into polyoma virions when cotransfected into mouse 3T6 cells with helper DNA from an early polyoma virus mutant. Herpes thymidine kinase activity was detected by a rapid in situ autoradiographic assay in which [125I]iododeoxycytidine was utilized as a substrate for the viral but not the cellular enzyme. (Author abstract) (15 Refs)

75. IDENTIFICATION OF PROTEINS ENCODED BY A FRAGMENT OF HERPES SIMPLEX VIRUS TYPE 2 DNA THAT HAS TRANSFORMING ACTIVITY.

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J Virol; 42(2):530-537 1982

Cloned BglII fragment N (map units 0.58 to 0.625) of herpes simplex virus Type 2 DNA has been shown to transform rodent cells to an oncogenic phenotype. RNA homologous to this fragment directs the synthesis of five polypeptides in a cell-free translation system. The approx mol wt of these proteins are 140,000, 61,000, 56,000, 35,000, and 23,500. The 35,000-dalton protein is the major species late in infection and is the only species detected before the onset of viral DNA replication. The arrangement of the sequences encoding these proteins along the herpes simplex virus Type 2 genome was determined by hybridization of the RNA to cloned PstI fragments of BglII-N and to single-stranded DNA segments cloned into M13mp7. Both the hybridization experiments and immunoprecipitation with monoclonal antibodies suggested that the 140,000-and 35,000-dalton proteins are at least partially colinear and share antigenic determinants. (Author abstract) (41 Refs)

76. CHANGE OF DNA NEAR THE ORIGIN OF REPLICATION ENHANCES THE TRANSFORMING CAPACITY OF HUMAN PAPOVAVIRUS BK.

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J Virol; 42(3):978-985 1982

A turbid-plaque-forming mutant (pm522) of human papovavirus BK, which has a small deletion at about 0.7 map unit and grows somewhat more slowly in human cells than does wild-

type BK virus, transformed hamster and rat cells in culture much more efficiently than did wild-type virus. Another plaque morphology mutant, pm525, forming turbid plaques larger than those of pm522 also had a high transforming capacity. The similar difference in transforming capability between wild-type and plaque morphology viruses was observed with DNAs extracted from virions. Recombinant viruses were constructed from the wild-type DNA fragment lacking HindIII-C (0.62 to 0.73 map unit) and pm522 HindIII-C (including the origin of replication) by the molecular cloning method. Characterization of the recombinants showed that the change near the origin of DNA replication was responsible both for the altered plaque morphology and for the enhanced transforming capacity of the BK virus mutant. (Author abstract) (33 Refs)

77. ECOTROPIC MURINE LEUKEMIA VIRUS DNA CONTENT OF NORMAL AND LYMPHOMATOUS TISSUES OF BXH-2 RECOMBINANT INBRED MICE.

Jenkins NA, Copeland NE, Taylor BA, Bedigian HG, Lee BK
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J Virol; 42(2):379-388 1982

BXH-2 recombinant inbred mice spontaneously produce a B-tropic murine leukemia virus (MuLV) beginning early in life and have a high incidence of non-T-cell lymphomas. These traits are not characteristic of the progenitor strains (C57BL/6J and C3H/HeJ) or of 11 other BXH recombinant inbred strains. Since B-tropic virus expression may be causally related to the high incidence of lymphoma in this strain, we have analyzed the ecotropic MuLV DNA content of both normal and lymphomatous tissues of BXH-2 mice. Southern analysis and hybridization with an ecotropic MuLV DNA-specific probe showed that DNA of normal BXH-2 tissues contained both parental N-tropic MuLV proviruses but lacked endogenous B-tropic MuLV DNA sequences. In addition, none of 116 F1 hybrid mice derived from male BXH-2 mice spontaneously produced ecotropic MuLV early in life. These results suggest that the B-tropic virus is horizontally transmitted in BXH-2 mice. Southern analysis of DNA from tumor tissues of 12 BXH-2 mice showed that amplification of ecotropic-specific DNA sequences had occurred in lymphomatous tissues of 3 mice and suggested that these tumors were monoclonal. The number of newly acquired proviruses, which appeared to be structurally nondefective and integrated at different sites, varied from one to three copies. Since lymphomatous tissues, from only 3 of 12 mice examined carried additional detectable ecotropic proviruses, these results suggest that amplification of ecotropic MuLV DNA sequences if not required for maintenance of transformation in BXH-2 lymphomas. (Author abstract) (36 Refs)

78. SITE OF PREMATURE TERMINATION OF LATE TRANSCRIPTION OF SIMIAN VIRUS 40 DNA: ENHANCEMENT BY 5,6-DICHLORO-1-BETA-D-RIBOFURANOSYLBENZIMIDAZOLE.

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Proc Natl Acad Sci USA; 79(9):2743-2747 1982

Sedimentation analysis of pulse-labeled RNA synthesized in nuclei isolated from simian virus 40-infected cells revealed an abundance of short cellular and viral RNAs. The reactive amount of the short chains is increased in nuclei isolated from cells treated with 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB). The short viral RNAs were purified by hybridization to and elution from simian virus 40 DNA on filters, and their sizes were determined by gel electrophoresis. A major band of 93-to 95-nucleotide-long RNA was observed along with additional minor bands. Identical bands were revealed when the viral RNA was purified from nuclei of cells pretreated with DRB. The major band was identified as an aborted transcript of a RNA that initiated at the major initiation site (nucleotide 243). We have found that the DNA region where the RNA stops is A + T rich and is immediately preceded by a G + C-rich region that exhibits dyad symmetry, resembling the termination signal in prokaryotes. These observations show that RNA polymerase II responds to the same termination signal as the prokaryotic enzyme and suggest that a mechanism of attenuation regulates simian virus 40 late transcription. (Author abstract) (47 Refs)

79. MAPPING OF AN ADENOVIRUS FUNCTION INVOLVED IN THE INHIBITION OF DNA DEGRADATION.

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J Virol; 42(3):969-977 1982

A function involved in the inhibition of DNA degradation has been assigned through complementation tests to a product of region E1b of the adenovirus genome (between 4.5 and 10.5 map units). DNA degradation induced by the adenovirus Type 12 (Ad12) cyt mutant H12cyt70 and the Ad5 early deletion mutant dl313 (with the deletion between 3.5 and 10.7 map units) was inhibited by coinfection with Ad5 region E1a (between 0 and 4.5 map units) mutants dl312 and hr1 and region E1b mutant hr6. The defect of inhibition of DNA degradation in Ad5 dl313 was also complemented in 293 cells. This DNase-inhibitory function does not appear to involve polypeptide IX or the 58,000-dalton polypeptide. Wild-type Ad12 induced DNA degradation in hamster embryo cells, suggesting that the DNase-inhibitory function is not expressed in these nonpermissive cells. Additional evidence suggests the involvement of a second viral product which positively influences the DNase activity and which appears to be an early function. (Author abstract) (47 Refs)

80. COMPLEMENTATION FOR REPLICATIVE FORM DNA REPLICATION OF A DELETION MUTANT OF H-1 BY VARIOUS PARVOVIRUSES.

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J Virol; 42(3):1118-1122 1982

A defective interfering genome of the parvovirus H-1 called dl1, has been isolated and characterized. The only alteration in dl1 that has been detected is a 300-base-pair deletion at map position 38. Replicative form DNA replication of dl1 requires the RF rep function of a helper virus and the parvoviruses H-1, H-3, MVM, or MVM(i), but not LuIII complement dl1 for replicative form DNA replication. (Author abstract) (8 Refs)

81. BIOLOGICAL AND BIOCHEMICAL STUDIES OF AFRICAN GREEN MONKEY LYMPHOTROPIC PAPOVAVIRUS.

Takemoto KK, Furuno A, Kato K, Yoshiike K
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J Virol; 42(2):502-509 1982

The growth of African green monkey lymphotropic papovavirus (LPV) in human lymphoblastoid cell line BJA-B was found to be slow and inefficient due to the accumulation of de-

fective particles. An analysis of molecularly cloned LPV DNAs showed that 3 of 19 clones had DNAs that were longer (5.1 kilobases) than the DNAs of the other clones. The 5.1-kilobase DNA was infectious for BJA-B cells, whereas the shorter (4.8-kilobase) molecules were defective. Unlike the wild-type virus, stocks of LPV made from cloned, infectious DNAs were homogenous and had higher titers. Using stocks of nondefective LPV, we investigated other biological properties. LPV replication in another human B-lymphoblastoid cell line was observed. The virus did not cause tumors when it was inoculated into newborn hamsters. Serological surveys of human and nonhuman primate sera indicated that virtually all primates, including humans, show evidence of infection by viruses antigenically related to LPV. (Author abstract) (29 Refs)

82. TWO DEFECTIVE DNAS OF HUMAN POLYOMAVIRUS JC ADAPTED TO GROWTH IN HUMAN EMBRYONIC KIDNEY CELLS.

Yoshiike K, Miyamura T, Chan HW, Takemoto KK
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J Virol; 42(2):395-401 1982

Human polyomavirus JC (JCV) adapted to growth in human embryonic kidney (HEK) cells contains two or more species of shorter-length viral DNA even after two cycles of plaque purification in HEK cells. We have molecularly cloned JCV DNA from one plaque isolate and determined the physical map of its DNA. Using gel electrophoresis and electron microscopy, we found that the cloned DNA consisted of two classes of JCV DNA. One class of DNA had a deletion of 30% (between 0.7 and 1.0 map units from the E. RI site) and an insertion of 1% at the same site in the late region. The other had a deletion of 35% (0.18 to 0.53 map units) and an insertion of 12% at the same site in the early region of the JCV genome. Both DNAs had added sequences near the origin of DNA replication. From their structure, the two DNAs appear to complement each other. These results indicate that the HEK-adapted JCV may be replicating by complementation between two defective mutants. (Author abstract) (25 Refs)

83. MOLECULAR CLONING OF THE FUJINAMI SARCOMA VIRUS GENOME AND ITS COMPARISON WITH SEQUENCES OF OTHER RELATED TRANSFORMING VIRUSES.

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J Virol; 42(3):1007-1016 1982

Full-length proviral DNA of Fujinami sarcoma virus (FSV) of chickens was molecularly cloned and characterized. An analysis of FSV DNA integrated in mammalian cells showed that restriction endonuclease SacI has a single cleavage site on FSV DNA. Unintegrated closed circular FSV DNA obtained from newly infected cells was linearized by digestion with SacI and cloned into lambdagtWES-lambdaB. The following three different molecules were isolated: FSV-1 (4.4 kilobases [kb]) and FSV-2 (4.7 kb), which appeared to be full-length FSV DNA molecules containing either one or two copies of the long terminal repeat structure, and FSV-3 (6 kb), which consisted of part FSV DNA and part DNA of unknown origin. An analysis of the structure of cloned FSV-1 and FSV-2 DNA molecules by restriction endonuclease mapping and hybridization with appropriate probes showed that about 2.6 kb of the FSV-unique sequence called FSV-fps is located in the middle of the FSV genome and is flanked by helper virus-derived sequences of about 1.3 kb at the 5' end and 0.5 kb at the 3' end. The long terminal repeats of FSV were found to have no cleavage site for either EcoRI or PvuI. Upon transfection, both FSV-1 DNA and FSV-2 DNA were able to transform mammalian fibroblasts. Four 32P-labeled DNA fragments derived from different portions of the FSV-fps sequence were used for hybridization to viral RNAs. We found that sequences within the 3' half of the FSV-fps gene are homologous to RNAs of PRCII avian sarcoma virus and the Snyder-Theilen strain of feline sarcoma virus, both of which were previously shown to contain transforming genes related to FSV-fps. These results suggest that the 3' portion of the FSV-fps sequence may be crucial for the transforming activity of fps-related oncogenic sequences. (Author abstract) (42 Refs)

84. CONSERVATION OF PROTEIN CODING POTENTIAL IN THE LONG TERMINAL REPEATS OF EXOGENOUS AND ENDOGENOUS MOUSE MAMMARY TUMOR VIRUSES.

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J Virol; 42(3):880-888 1982

In vitro protein synthesis and DNA sequence analysis indicate that mouse mammary tumor virus differs from other well-characterized retroviruses in that the long terminal repeat region of the provirus has the capacity to encode proteins. Different exogenously transmitted mouse mammary tumor virus strains and endogenous proviral units conserved this open reading frame feature in the long terminal repeat despite a variation in nucleotide sequence. The proteins encoded by the different long terminal repeats were clearly related, but showed minor variations in size and tryptic peptide maps. In each case, the largest in vitro product had a mol wt of about 36,000 to 37,000, suggesting that the open reading frame sequences must extend for approx 1,000 nucleotides beginning at the extreme 5' end of the long terminal repeat. The fact that the reading frame was conserved among these viruses argues in favor of an in vivo function for the open reading frame protein. (Author abstract) (29 Refs)

85. RESTRICTION MAPPING AND PHYSICAL CHARACTERIZATION OF THE COTTONTAIL RABBIT PAPILLOMAVIRUS GENOME IN TRANSPLANTABLE VX2 AND VX7 DOMESTIC RABBIT CARCINOMAS.

Favre M, Jibard N, Orth G
Unite 190 de l'Institut National de la Sante et de la Recherche Medicale, Unite des Papillomavirus, Institut Pasteur, Paris, France
Virology; 119(2):298-309 1982

The transplantable VX2 and VX7 carcinomas were analyzed for the presence, the nature, and the physical state of cottontail rabbit papillomavirus (CRPV) DNA sequences. Two CRPV DNA preparations, obtained from pooled cottontail rabbit papillomas, were found highly homogeneous as to their sensitivities to seven restriction endonucleases and were, therefore, considered as wild-type CRPV DNA. A cleavage map of the wild-type genome was constructed by locating 14 restriction sites. The carcinoma cells contained multiple copies of apparently whole viral genomes (42 and 444 genome equivalents per diploid VX2 and VX7 cell DNA contents, respectively), as determined by reassociation kinetic experiments and blot hybridization analyses of restricted VX2 and VX7 cell DNAs. Viral DNA molecules bearing a deletion of about 10% of the genome length, as well as minor sequence rearrangements, were also detected in VX7 carcinoma cells (about 25 copies

per diploid cell DNA content). In both VX2 and VX7 cell DNAs, either unrestricted or treated with a no-cut endonuclease for the CRPV genome (PvuI), viral DNA sequences were present exclusively as slow-migrating species with similar electrophoretic mobilities. The conversion of these species into one-genome-unit-length linear DNA molecules, by a one-cut endonuclease for both wild-type and deleted DNA molecules (SmaI), shows that the viral sequences are not integrated in the cell genome. After cleavage of VX7 cell DNA by a no-cut endonuclease for deleted DNA molecules (EcoRI), the deleted molecules migrated with a mobility corresponding to form I and II DNA molecules. This suggests that the viral genomes may be present in carcinoma cells as free catenated DNA molecules. (Author abstract) (42 Refs)

86. NUCLEOTIDE SEQUENCE OF THE TRANSFORMING GENE OF SIMIAN SARCOMA VIRUS.

Devare SG, Reddy EP, Robbins KC, Andersen PR, Tronick SR, Aaronson SA
Lab. Cellular and Molecular Biology, Natl. Cancer Inst., Bethesda, MD, 20205
Proc Natl Acad Sci USA; 79(10,part1):3179-3182 1982

The sequence of the transforming region of simian sarcoma virus (SSV) has been determined by using molecularly cloned viral DNA. This region encompassed the 1.0-kilobase pair woolly monkey cell-derived insertion sequence, v-sis, and flanking simian sarcoma-associated viral (SSAV) sequences. A 675-nucleotide-long open reading frame commenced 19 nucleotides within the SSAV sequences to the left of the v-sis helper viral junction and terminated within v-sis itself. Possible promoter and acceptor splice signals were detected in helper viral sequences upstream from this open reading frame, and potential polyadenylylation sites were identified downstream both within v-sis and in helper viral sequences beyond v-sis. The recombinational event that led to the generation of SSV occurred in the middle of two functional codons, indicating that SSAV provided the regulatory elements for transcription as well as the initiation codon for translation of SSV cell-derived transforming sequences. (Author abstract) (28 Refs)

87. NUCLEOTIDE SEQUENCE ANALYSIS OF THE LONG TERMINAL REPEAT OF INTEGRATED SIMIAN SARCOMA VIRUS: EVOLUTIONARY RELATIONSHIP WITH OTHER MAMMALIAN RETROVIRAL LONG TERMINAL REPEATS.

Devare SG, Reddy EP, Law JD, Aaronson SA
Lab. Cellular and Molecular Biology, NCI, Bethesda, MD, 20205
J Virol; 42(3):1108-1113 1982

Nucleotide sequence analysis of the long terminal repeat (LTR) of the integrated simian sarcoma virus showed that the simian sarcoma virus LTR comprised 504 nucleotides with an inverted repeat of seven bases at its 5' and 3' termini. At the site of simian sarcoma virus integration, cellular flanking sequences adjacent to the proviral LTR contained a direct repeat of four bases. A 13-base sequence after the 5' LTR was found to be complementary to prolyl tRNA, suggesting that tRNA-Pro may serve as the primer for reverse transcription of simian sarcoma virus RNA. The U5 and R regions, derived respectively from the 5' end and terminally redundant sequences of the viral RNA, were found to have similar organization and sequence homology close to that of Moloney murine sarcoma virus or Moloney murine leukemia virus. These results indicate that regions within LTRs with known functionally important sequences have been most well conserved during retrovirus evolution. (Author abstract) (45 Refs)

88. NUCLEOTIDE SEQUENCE ANALYSIS OF THE LONG TERMINAL REPEAT OF AVIAN MYELOBLASTOSIS VIRUS AND ADJACENT HOST SEQUENCES.

Rushlow KE, Lautenberger JA, Reddy EP, Souza LM, Baluda MA, Chirikjian JG, Papas TS
(c/o Papas) Lab. Molecular Oncology, NCI, Bethesda, MD, 20205
J Virol; 42(3):840-846 1982

The nucleotide sequence of the integrated avian myeloblastosis virus long terminal repeat has been determined. The sequence is 385 base pairs long and is present at both ends of the viral DNA. The cell-virus junctions at each end consist of a six-base-pair direct repeat of cell DNA next to the inverted repeat of viral DNA. The long terminal repeat also contains promoter-like sequences, an mRNA capping site, and polyadenylation signals. Several features of this long terminal repeat suggest a structural and functional similarity with sequences of transposable and other genetic elements. Comparison of these sequences with long terminal repeats of other avian retroviruses indicates that there is a great variation in the 3' unique sequence (U3), whereas the 5' specific sequences (U5) and the R region are highly conserved. (Author abstract) (31 Refs)

89. DEMONSTRATION OF BIOLOGICAL ACTIVITY AND NUCLEOTIDE SEQUENCE OF AN IN VITRO SYNTHESIZED CLONE OF THE MOLONEY MURINE SARCOMA VIRUS MOS GENE.

Donoghue DJ
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
J Virol; 42(2):538-546 1982

A clone of the Moloney murine sarcoma virus mos gene derived by in vitro reverse transcription was characterized. When assayed for focus formation by DNA transfection on NIH/3T3 cells, this clone was biologically inactive, presumably due to the absence of a long terminal repeat sequence. Therefore, a long terminal repeat was inserted into the clone by in vitro recombination, after which the mos gene was able to transform NIH/3T3 cells efficiently. The nucleotide sequence encompassing the transforming region of this clone was determined. A single long open reading frame was observed, which potentially encodes a polypeptide of 41,000 daltons. This open reading frame initiates with the first five amino acids of the murine leukemia virus env gene, after which it enters the mos sequence, where it terminates. The nucleotide sequence described in this paper was compared with other sequences of mos in an effort to resolve discrepancies in the position of the long open reading frame. Although Moloney murine sarcoma virus retains the 3' splicing site of the murine leukemia virus env gene, a mos-specific mRNA which corresponds structurally to the murine leukemia virus env mRNA was not identified. The sequence described here revealed a single nucleotide change in the proposed env gene 3' splicing site which was retained in Moloney murine sarcoma virus. This deviation from the consensus 3' splicing sequence may underlie the absence of mos expression via the env gene splicing pathway. (Author abstract) (44 Refs)

90. NUCLEOTIDE SEQUENCE OF THE AKV ENV GENE.

Lenz J, Crowther R, Straceski A, Haseltine W
(c/o Haseltine), Sidney Farber Cancer Inst., Boston, MA, 02115
J Virol; 42(2):519-529 1982

The sequence of 2,191 nucleotides encoding the env gene of murine retrovirus Akv was determined by using a molecular clone of the Akv provirus. Deduction of the encoded amino

acid sequence showed that a single open reading frame encodes a 638-amino acid precursor to gp70 and p15E. In addition, there is a typical leader sequence preceding the amino terminus of gp70. The locations of potential glycosylation sites and other structural features indicate that the entire gp70 molecule and most of p15E are located on the outer side of the membrane. Internal cleavage of the env precursor to generate gp70 and p15E occurs immediately adjacent to several basic amino acids at the carboxyl terminus of gp70. This cleavage generates a region of 42 uncharged, relatively hydrophobic amino acids at the amino terminus of p15E, which is located in a position analogous to the hydrophobic membrane fusion sequence of influenza virus hemagglutinin. The mature polypeptides are predicted to associate with the membrane via a region of 30 uncharged, mostly hydrophobic amino acids located near the carboxyl terminus of p15E. Distal to this membrane association region is a sequence of 35 amino acids at the carboxyl terminus of the env precursor, which is predicted to be located on the inner side of the membrane. By analogy to Moloney murine leukemia virus, a proteolytic cleavage in this region removes the terminal 19 amino acids, thus generating the carboxyl terminus of p15E. This leaves 15 amino acids at the carboxyl terminus of p15E on the inner side of the membrane in a position to interact with virion cores during budding. The precise location and order of the large RNase T1-resistant oligonucleotides in the env region were determined and compared with those from several leukemogenic viruses of AKR origin. This permitted a determination of how the differences in the leukemogenic viruses affect the primary structure of the env gene products. (Author abstract) (54 Refs)

OTHER RELATED STUDIES

91. THE CONSTRUCTION OF COSMID LIBRARIES OF EUKARYOTIC DNA USING THE HOMER SERIES OF VECTORS.

Chia W, Scott MR, Rigby PW
Cancer Res. Campaign, Eukaryotic Molecular Genetics Res. Group, Imperial Coll. Science and Technology, London SW7 2AZ, England
Nucleic Acids Res; 10(8):2503-2520 1982

We have constructed and characterized a series of approved, disabled cosmid vectors which we call Homer cosmids and have examined the optimal conditions for the construction of libraries of eukaryotic DNA segments using these vectors. Analysis of these libraries shows that most of the sequences we have tested for are present at the expected frequency and that the libraries can be stably propagated. We have also directly tested the stability of cosmid clones carrying tandemly repeated inserts. This work shows that it should be possible to clone most eukaryotic genes using cosmid vectors and that such cloning systems have considerable advantages over those more commonly used. (Author abstract) (51 Refs)

92. SELECTIVE ALKYLATION OF NUCLEIC ACIDS BY CARCINOGENS MAY CAUSE MISCODING IN DNA. A THEORETICAL STUDY.

Klopman G, Ray A
Case Western Reserve Univ., Cleveland, OH, 44106
Cancer Biochem Biophys; 6(1):31-35 1982

The CNDO/II method was used to study the pairing abilities of various alkylated nucleic acids. On the basis of these results, a mechanism for mutation (carcinogenesis) is proposed. Our calculations suggest that O-(6) alkylated guanine and/or O-(4) alkylated thymine could cause miscoding during the replication of DNA. (Author abstract) (26 Refs)

93. INDUCTION OF PROLACTIN-DEFICIENT VARIANTS OF GH3 RAT PITUITARY TUMOR CELLS BY ETHYL METHANESULFONATE: REVERSION BY 5-AZACYTIDINE, A DNA METHYLATION INHIBITOR.

Ivarie RD, Morris JA
Dept. Molecular and Population Genetics, Univ. Georgia, Athens, GA, 30602
Proc Natl Acad Sci USA; 79(9):2967-2970 1982

GH3 cells are a rat pituitary tumor line expressing two pituitary peptide hormones, prolactin (rPRL) and growth hormone. Recently, it was found that the DNA alkylating agent ethyl methanesulfonate can induce the appearance of rPRL-deficient GH3 cell variants at a high frequency (ca 20-30%). As shown here, such variants cannot be induced at high frequency by irradiation of wild-type GH3 cells with ultraviolet light, indicating that the effect may be specific to treatment with alkylating agents. Furthermore, the DNA methylation inhibitor 5-azacytidine reverted an ethyl methanesulfonate-induced rPRL-deficient variant into rPRL-expressing cells at high frequency (ca 50%). The revertants were stable for at least 30-35 generations. These results support the hypothesis that the alkylating agent may promote the specific methylation of the rPRL gene or a gene regulating its activity, either one of which leads to inactivation of expression of the rPRL gene in GH3 cells. (Author abstract) (52 Refs)

94. RADIOBIOLOGY OF A DIFFERENTIATING CELL SYSTEM IN VITRO.

Borsa J, Ewing D, Dugle DL, Einspenner M
Medical Biophysics Branch, Whiteshell Nuclear Res. Establishment, Atomic Energy Canada Res. Company, Pinawa, Manitoba, Canada ROE 1LO
Int J Radiat Biol; 41(4):369-388 1982

Friend erythroleukemia cells (FELC) in vitro were used to examine the effects of ionizing radiation on differentiation and proliferation of mammalian cells. Results suggest that x-rays can affect differentiation in two different ways. First, x-rays inactivate the ability of these cells to respond to an external trigger of differentiation, eg, dimethyl sulfoxide. Second, x-rays themselves trigger a partial differentiation response, in the absence of any other external trigger. The radiation-induced lesions leading to these two end-points are not repaired in split-dose experiments, unlike those lesions which lead to loss of cell proliferative capacity. The profile of soluble FELC proteins, as analyzed by isoelectric focusing, was also affected by irradiation. These effects of ionizing radiation on the expression of genetic information in mammalian cells have important implications for radiobiology, particularly at low doses where acute lethal effects are minimal. (Author abstract) (14 Refs)

95. RELATIONSHIP BETWEEN THE PROLIFERATING ACTIVITY AND THE RESPONSE OF MOUTH TUMORS TO RADIOTHERAPY.

Karakulov RK, Poliakov PIu, Pelevina II, Kiseleva ES, Dar'ialova SL, Saenko AS
Inst. Chemical Physics, Moscow, USSR
Med Radiol (Mosk); 27(3):16-23 1982

The effect of the kinetic parameters of cell proliferation on the efficacy of radiotherapy (RT) was studied in 11 patients

with Stage II-III squamous cell carcinoma of the mouth and oropharynx. The patients received modified fractionated RT: 400 rads/day during the first 3 days, 100 rads, bid during the next 3-10 days up to a total focal dose of 2,600-3,000 rads. After a 7-15-day interval, the irradiations were repeated to a total focal dose of 5,000-6,000 rads. Biopsy specimens were obtained prior to the RT and after application of 1,200 rads. The mitotic index in nonirradiated tumors ranged from 8.0% to 22.5%. The DNA-polymerase index ranged from 19% to 33.2%. Significant (3-5-fold) decrease of MI after irradiation with 1,200 rads was correlated with tumor regression after completion of RT. Similarly, a 4-10-fold decrease of the fraction of proliferating cells coincided with complete tumor regression. Tumor regression did not depend upon the MI or the fraction of proliferating cells prior to the RT. (21 Refs) (In RUS)

96. DIFFERENTIAL CYTOLOGICAL DIAGNOSIS OF SQUAMOUS CELL CARCINOMA OF THE LOWER LIP AND PEMPHIGUS VULGARIS.

Tiurbeeva ML, Shmarina TI
Res. Oncological Inst., Rostov, USSR
Vestn Dermatol Venerol; (3):49-51 1982

Cytological examination of tumor specimens from 96 patients with clinical diagnosis of the squamous cell carcinoma (SCC) of the lower lip showed pemphigus vulgaris in 3 (1 man, 2 women; 53-66 yr old). Characteristic cytological features of SCC included the layers and clusters of atypical and polymorphic cells with clear slightly basophilic cytoplasm and large nuclei with irregular contour and 1-3 nucleoli. The specimens of pemphigus vulgaris contained the acantholytic cells with increased DNA content. (13 Refs) (In RUS)

97. STRUCTURE-CYTOTOXICITY RELATIONSHIPS OF NITROIMIDAZOLES IN AN IN VITRO SYSTEM.

Edwards DI, Knox RJ, Knight RC
Chemotherapy Res. Unit, Dept. Paramedical Sciences, North East London Polytechnic, Romford Road, London E15 4LZ, England
Int J Radiat Oncol Biol Phys; 8(3/4):791-793 1982

The nitrite released from the reduced nitroimidazoles (NIM) in the absence and the presence of DNA was studied to investigate the nature of the active species responsible for the cytotoxic effect of NIM. If cytotoxicity is expressed as the reduced drug-nucleotide ratio which produces a 10% release of thymidine from DNA, then a linear correlation is obtained between the DNA damage and the one-electron redox potential of 10 NIM. The correlation was the same as that found for the cytotoxicity of NIM against Bacteroides fragilis measured as the minimum inhibitory concentration. (12 Refs)

98. INDUCTION OF IN VITRO PROLIFERATION AND MATURATION OF HUMAN ANEUPLOID MYELOGENOUS LEUKEMIC CELLS.

Chiao JW, Andreeff M, Freitag WB, Arlin Z
Sloan-Kettering Inst. Cancer Res., New York, NY, 10021
J Exp Med; 155(5):1357-1369 1982

Terminal maturation of aneuploid leukemic cells from patients with acute myelogenous leukemia was induced by supplementation of media with lymphocyte-conditioned medium (CM). Cells in culture suspensions underwent changes in morphology, and some cell aggregates were detected. As the proportion of monocyte- and macrophage-like cells increased with time, the proportion of immature cells decreased. Flow cytometric measurement of cells during the maturation-induction period showed an appearance of cell populations having lower cellular RNA content than populations not treated with conditioned medium. CM-induced maturation of leukemic cells to monocyte- and macrophage-like cells appears to be independent of DNA abnormality. In three patients studied, hyperdiploid DNA stem lines were found in all cells throughout the culture period. Macrophage-like cells with mature functional and marker features had the same aneuploid stem line as the undifferentiated cells, establishing the leukemic origin of the differentiated cells. Because of the higher rate of proliferation in the CM-supplemented cultures than in those lacking CM, interaction of CM with cells seems to have resulted in stimulation of some cells from the resting to the replicating phase. (22 Refs)

99. POLYPLOID RAT GLIOMA CELLS. PRODUCTION, OSCILLATIONS OF MEMBRANE POTENTIAL AND RESPONSE TO NEUROHORMONES.

Heumann R, Reiser G, Van Calker D, Hamprecht B
Max-Planck-Institut fur Psychiatrie, 8033 Martinsried, W. Germany
Exp Cell Res; 139(1):117-126 1982

Due to their small size, electrophysiological investigation of C6 rat glioma cells by intracellular recording with microelectrodes is very difficult. In order to facilitate such electrophysiological studies, the degree of ploidy of C6 cells was increased in three successive steps. At each step complementary drug-resistant mutants of C6 were fused and hybrid cell clones isolated in selective culture medium. Cell volume was measured by electronic cell sizing and DNA content per cell assessed by impulse cytofluorimetry. In the final polyploid line, C6-4-2, both parameters were found to be increased six-fold in comparison with the original cell line C6. In response to beta-adrenergic agonists and prostaglandin E1 the intracellular concentration of cAMP was raised in C6-4-2 cells. Stable membrane potentials could be recorded from the polyploid glioma cells much more easily than from the original C6 cells. The plasma membrane of the C6-4-2 cells could hyperpolarize spontaneously with the a frequency of about 1 cycle/min. Elevation of the concentration of Ca++ ions (but not other earth alkali ions) in the medium induced the onset of the spontaneous hyperpolarizations. The membranes of the C6-4-2 cells also hyperpolarized in response to acetylcholine, gamma-aminobutyric acid (GABA) and glutamate. (Author abstract) (51 Refs)

100. CYTOCHEMICAL GRADING OF THE MALIGNANCY OF BREAST TUMORS.

Auer G, Caspersson T
Institution for Tumorpatologi, Karolinska institutet, S-104 01 Stockholm, Sweden
Lakartidningen; 79(20):2018-2020 1982

Correlations between the DNA distribution patterns of the tumor cell nuclei, determined by cytophotometric method, and survival were studied based on tumor biopsy reports and specimens from approx 200 women with confirmed breast cancer, who were followed up for approx 15 yr. DNA levels within the limits established for normal diploid and tetraploid cells, corresponding to very slow cell growth, were found in specimens from patients with good prognosis, ie, with longer survival. Wide spread of the DNA levels, at times even beyond the limits characteristic of normal tissue, which correspond to rapidly growing cells, were found in specimens from patients with poor prognosis. The findings indicate a clear correlation between the DNA pattern and individual survival, and the

DNA cytophotometry can be a useful tool in the assessment of the prognosis of breast cancer patients. (5 Refs) (In SWE)

101. FEULGEN-HYDROLYSIS PROFILES IN CELLS EXFOLIATED FROM THE CERVIX UTERI: A POTENTIAL AID IN THE DIAGNOSIS OF MALIGNANCY.

Millett JA, Husain O, Bitensky L, Chayen J
(c/o Husain) Dept. Cytopathology, Charing Cross Hosp., Fulham Palace Road, London W6 8RF, England
J Clin Pathol; 35(3):345-349 1982

By varying the time of hydrolysis for the Feulgen reaction, done under conditions that protect the backbone of the DNA, it is possible to distinguish three species of DNA that are characterized by their lability to acid hydrolysis. The most labile DNA was found, in greatest proportions, in malignant cells; this may be helpful in diagnostic cytology. The fact that the cytologically normal cells, in Grade V smears, also show this labile DNA may well facilitate cytological screening even in those smears that contain very few neoplastic cells. (Author abstract) (21 Refs)

102. THE SIGNIFICANCE OF PLOIDY AND PROLIFERATION IN THE CLINICAL AND BIOLOGICAL EVALUATION OF BLADDER TUMORS: A STUDY OF 100 UNTREATED CASES.

Tribukait B, Gustafson H, Esposti PL
Dept. Medical Radiobiology, Karolinska Institutet, S-104 01, Stockholm, Sweden
Br J Urol; 54(2):130-135 1982

Cell material from 100 cases of newly diagnosed bladder tumors was studied with rapid flow cytofluorometric DNA analysis. The degree of ploidy and the percentage of cells in the S-phase characterizing the proliferation pattern of the tumors were determined from DNA histograms. The results were related to the tumor categories and to the histopathological grading of the tumors. Approximately 40% of T1 tumors showed aneuploid DNA patterns, while in the other categories aneuploidy was found in almost all cases. With few exceptions, tumors of Grade 1 were found to be diploid and tumors of Grade 3 aneuploid. In tumors of Grade 2, two-thirds were diploid and one-third aneuploid. In the group of aneuploid tumors the tetraploid cases exhibited the least malignant characteristics as judged by histological grades and tumor categories. The aneuploid non-tetraploid tumors increased in malignancy the more they deviated from tetraploidy. This might be explained by our observations of a positive correlation between the degree of ploidy and the degree of proliferation. The proliferation increased from 10% in tetraploid tumors to 20% in triploid and pentaploid tumors. (Author abstract) (14 Refs)

103. FORM AND FUNCTION OF RETROVIRAL PROVIRUSES.

Varmus HE
Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143
Science; 216(4548):812-820 1982

Retroviruses have proved to be useful reagents for studying genetic and epigenetic (such as regulatory) changes in eukaryotic cells, for assessing functional and structural relationships between transposable genetic elements, for inducing insertional mutations, including some important in oncogenesis, and for transporting genes into eukaryotic cells, either after natural transduction of putative cellular oncogenes or after experimental construction of recombinant viruses. Many of these properties of retroviruses depend on their capacity to establish a DNA (proviral) form of their RNA genomes as a stable component of host chromosomes, in either somatic or germinal cells. (Author abstract) (114 Refs)

104. ISOLATION OF A RETROVIRUS FROM CULTURED EQUINE SARCOID TUMOR CELLS.

Cheevers WP, Roberson SM, Brassfield AL, Davis WC, Crawford TB
Dept. Veterinary Microbiology and Pathology, Washington State Univ., Pullman, WA, 99164
Am J Vet Res; 43(5):804-806 1982

A virus with the morphologic and biochemical properties of the family Retroviridae has been isolated from cultured cells explanted from a malignant tumor induced by intradermal inoculation of equine sarcoid cells into a combined immunodeficient Arabian foal. By electron microscopy, intracytoplasmic, extracellular, and budding particles measuring 89 to 120 nm with electron-lucent cores were seen. Virus purified from the medium of cultured cells had a buoyant density of 1.15 g/cm3 in isopycnic sucrose-gradient centrifugation, incorporated radiolabeled uridine but not thymidine, and had constitutive RNA-dependent DNA polymerase which required Mn++ for optimal endogenous activity. (Author abstract) (18 Refs)

105. ANALYSIS OF EPSTEIN-BARR VIRUS (EBV) OF P3HR-1: ISOLATION OF EBV WITH EBNA INDUCTION ABILITY IN HUMAN CORD LYMPHOCYTES AND WITHOUT EA INDUCTION ABILITY IN RAJI CELLS.

Yano S, Tanaka A, Takada K, Fujiwara S, Osato T, Nonoyama M
(c/o Nonoyama) Dept. Virology, Showa Univ. Res. Inst. Biomedicine Florida, 5180 113th Ave. N., Clearwater, FL, 33520
Virology; 119(2):392-398 1982

A subline of P3HR-1 cells was isolated through a prolonged (over 1 yr) propagation of the cells at a non-EBV-productive condition followed by cell cloning procedures. Cloned cells thus obtained, designated DHR1, produced EBV when brought back to the EBV-productive condition. Restriction enzyme analysis of the viral DNA revealed that DHR1 EBV is composed of an apparently homogeneous EBV population, and it displays a similar but not identical genome organization compared with HR-1 EBV. The characteristic biological properties of DHR1 EBV included the ability to induce EBV-associated nuclear antigen (EBNA) in human cord lymphocytes and the inability to induce EBV-associated early antigen (EA) in Raji cells. These are in striking contrast to the behavior of the parental HR-1 EBV. Thus, P3HR-1 cultures after a period of nonproductivity reinitiated the production of virus with an apparently homogeneous and unique population of EBV distinguishable from the original HR-1 virus. (Author abstract) (18 Refs)

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MOLECULAR BIOLOGY

DNA

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MECHANISMS OF DNA REPLICATION

1. CONCENTRATION DEPENDENT ALTERATIONS OF DNA REPLICATION INITIATION AND ELONGATION BY BENZO[A]PYRENE DIOL EPOXIDE.

Bowden GT, McGovern V, Ossanna N, Rosenthal H
Div. Radiation Oncology, Dept. Radiology, Univ. Arizona Medical Sch., Tucson, AZ, 85724
Carcinogenesis; 3(5):473-480 1982

Vero cells treated with various concentrations of (+-)7beta,8alpha-dihydroxy-9alpha,10alpha-epoxy, 7,8,9,10-dihydrobenzo[a]pyrene (BP-diol epoxide I) exhibited dose-dependent inhibition in both the rate of DNA synthesis and in the size of nascent DNA. The max inhibition was seen 2-3 hr after addition of BP-diol epoxide I. A recovery in both the rate of synthesis and size of nascent DNA was observed 5-10 hr after treatment. The pH step alkaline elution assay which separates different nascent DNA replication intermediates was used to investigate whether the inhibition and recovery noted above could be accounted for by alterations in DNA replication initiation (DNA synthesis within a replicon) or elongation (rejoining of replicons). At lowest dose studied (0.166 uM BP-diol

epoxide I) a reversible inhibition in DNA initiation was observed. At the higher dose levels (0.66 uM and 1.66 uM BP-diol epoxide I) inhibition of both DNA initiation and elongation were observed and inhibition of elongation predominated. The inhibition in elongation was detected by an increase in the relative amount of low mol wt nascent DNA associated with DNA synthesis within a replicon and a relative decrease in the higher mol wt elongated DNA. A reversal in the inhibition of both initiation and elongation was noted. (Author abstract) (26 Refs)

2. SYNTHESIS OF MURINE LEUKEMIA VIRUS PLUS STRONG STOP DNA INITIATES AT A UNIQUE SITE.

Mitra SW, Chow M, Champoux J, Baltimore D
Dept. Biochemical Genetics, Merck Sharp and Dohme Res. Lab., Rahway, NJ, 07065
J Biol Chem; 257(11):5983-5986 1982

The 5'-terminal segment of plus strong stop DNA has been isolated from an endogenous virion reverse transcription reaction and characterized. The plus strong stop DNA is shown to initiate with the sequence 5'AATGAAAGA. The 5'-terminal nucleotide is a deoxyribonucleotide. No residual protein or RNA moiety was found attached to the plus strong stop DNA. Thus, the mechanism which primes plus strand synthesis is still unclear. No evidence for multiple initiation sites or heterogeneous starts is observed. This data indicates that the initiation of plus strong stop DNA synthesis by the virion RNA-dependent DNA polymerase is a precise event which occurs at a unique site on the retrovirus genome. (Author abstract) (19 Refs)

EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

3. METABOLISM AND THERAPEUTIC EFFICACY OF 9-BETA-D-ARABINOFURANOSYL-2-FLUOROADENINE AGAINST MURINE LEUKEMIA P388.

Avramis VI, Plunkett W
(c/o Plunkett) Dept. Developmental Therapeutics, Univ. Texas System Cancer Center, M. D. Anderson Hosp. and Tumor Inst., Houston, TX, 77030
Cancer Res; 42(7):2587-2591 1982

The biochemical basis for the differential therapeutic activity of equally toxic doses of 9-beta-D-arabinofuranosyl-2-fluoroadenine (F-ara-A) administered on two schedules to tumor-bearing mice has been studied. A single dose (234 mg/kg) of F-ara-A in solution decreased the number of P388 leukemia cells by greater than 10(3), whereas a multiple-dose regimen (41 mg/kg every day for 5 days) of equal toxicity to the host was ineffective at reducing the tumor burden. No antitumor activity was seen when an equal dose of the relatively insoluble F-ara-A was injected as a suspension. The 5'-triphosphate of F-ara-A accumulated in P388 cells in levels proportional to the dose of the nucleoside and disappeared from these cells at an exponential rate with a half-life of 2.9 hr, which was independent of the cellular concentration of the nucleotide. The extent and duration of the inhibition of DNA synthesis of P388 cells was dependent on the dose of F-ara-A, but the rates of recovery were similar and in proportion to the cellular concentration of the analog triphosphate. The extent of the inhibition of DNA synthesis in host bone marrow and intestinal mucosa was also related to the dose of F-ara-A, but the recovery of these tissues proceeded to similar, incomplete levels (less than 60% of initial) 24 hr after F-ara-A injection of either 41 or 234 mg/kg. These results suggest that the equal toxicity of the two regimens of F-ara-A may be attributed to the similar extent of inhibition of host-tissue DNA synthesis evoked by each. In contrast, the greater extent and longer duration of inhibition of P388 cell DNA synthesis caused by the single dose of F-ara-A was responsible for its superior therapeutic activity. Measurements of F-ara-A triphosphate concentrations and the DNA-synthetic capacity of tumor and host tissues are determinants of the action of F-ara-A and may be used to predict optimal therapeutic dose schedules. (Author abstract) (23 Refs)

4. MODE OF ACTION OF 5-FLUOROCYTIDINE AND 5-FLUORO-2'-DEOXYCYTIDINE IN L5178Y CELLS IN VITRO.

Yoshida M, Hoshi A, Kuretani K, Saneyoshi M
Natl. Cancer Res. Inst., Tsukiji 5-1-1, Chuo-ku, Tokyo 104, Japan
Chem Pharm Bull (Tokyo); 30(3):1018-1023 1982

The mode of antiproliferative action of 5-fluorocytidine and 5-fluoro-2'-deoxycytidine was examined by studying incorporation of labeled deoxycytidine, thymidine, L-leucine, and uridine, and its reversal in mouse leukemia L5178Y cells in vitro. These nucleosides apparently inhibit DNA synthesis by blocking thymidylate synthetase, presumably through formation of 5-fluoro-2'-deoxyuridine monophosphate after deamination by deoxycytidylate deaminase in the pyrimidine de novo pathway. (20 Refs)

5. INCREASED RELATIVE EFFECTIVENESS OF DOXORUBICIN AGAINST SLOWLY PROLIFERATING SARCOMA 180 CELLS AFTER PROLONGED DRUG EXPOSURE.

Ritch PS, Occhipinti SJ, Skramstad KS, Shackney SE
Hematology-Oncology Section, Dept. Medicine, Medical Coll. Wisconsin, 8700 W. Wisconsin Ave., Milwaukee, WI, 53226
Cancer Treat Rep; 66(5):1159-1168 1982

Doxorubicin (ADR) lethality was studied in Sarcoma 180 cells grown in vitro during log phase and early plateau phase. For both log phase and plateau phase cells, ADR lethality increased progressively with continuous drug exposure, and was dependent on drug concentration at every point in time. For any given level of toxicity to log phase cells, greater killing of plateau phase cells was achieved with prolonged drug exposure. The inhibition of DNA synthesis by ADR was delayed in onset, and increased gradually, both at low and high drug concentrations. The data suggest that the dependence of ADR lethality on proliferation rate and the higher therapeutic ratio in plateau phase cells with prolonged drug exposure are not directly related to the ADR effects on DNA synthesis. (Author abstract) (36 Refs)

6. INHIBITION OF SEMICONSERVATIVE DNA SYNTHESIS IN ICR 2A FROG CELLS EXPOSED TO MONOCHROMATIC UV WAVELENGTHS (252-313 NM) AND PHOTOREACTIVATING LIGHT.

Rosenstein BS
Dept. Radiology, Univ. Texas Health Science Center, 5323 Harry Hines Blvd., Dallas, TX, 75235
Radiat Res; 90(3):509-517 1982

The role of pyrimidine dimers and other photoproducts in inhibition of DNA synthesis in UV-irradiated ICR 2A cells also exposed to photoreactivating light (PRL) was investigat-

ed. Exposure of cells to 265-and 289-nanometer (nm) radiation caused an inhibition of DNA synthesis that was greatly reduced if cells were subsequently treated with PRL. The effect of dimer removal was less in cells exposed to 302-nm radiation and was nearly eliminated for about the first 12 hr after 313-nm irradiation. In contrast to UV, the inhibition resulting from exposure to gamma irradiation reached a max 2-6 hr after treatment, at which time the rate of DNA synthesis began to increase. At every wavelength tested (seven wavelengths between 252-313 nm), the rate of DNA synthesis was higher in UV-irradiated cells exposed to PRL compared with cells incubated in the dark. For each wavelength, exposure of UV-irradiated cells to PRL resulted in dimer removal which was accompanied by increased DNA synthesis and survival. The action spectrum for DNA synthesis inhibition was similar to the absorption spectrum of DNA and the action spectra for cell killing and UV-endonuclease-sensitive site induction. These findings indicate that (1) dimers act at lesions induced by 252-313-nm irradiation that cause inhibition of semiconservative DNA synthesis, and (2) nondimer lesions become increasingly important in DNA inhibition resulting from exposure of cells to UV greater than 300-nm. (29 Refs)

7. INTERFERON INHIBITS PWM INDUCED B CELL DIFFERENTIATION BUT NOT ONSET OF PROLIFERATION.

Yen A, Lewin D, Fairchild D
Dept. Physiology and Biophysics, Univ. Iowa, Basic Science Buliding, Iowa City, IA, 52242
Leuk Res; 6(2):221-229 1982

Results of a test, using pokeweed mitogen (PWM) driven human lymphocyte system to elicit an in vitro humoral response are reported. The net DNA synthesis by PWM stimulated cells was reduced by approx 40% in the presence of interferon (INF) after 96 and 144 hr of culture. The presence of INF resulted in reduced net synthesis of DNA, indicating reduced net cell proliferation due to INF. The distribution of cells with respect to the G1/0-, S-, and G2 + M-cell cycle phases was similar in PWM stimulated cell cultures with or without INF. INF treated cells initiated DNA synthesis at the same time as untreated cells. The presence of INF, thus, did not affect the minimum transit from G0 to S. No difference in the population size was observed for cells in the presence or absence of INF until after 48 hr. However, the size of INF treated populations thereafter was persistently less. This is consistent with the INF induced reduced proliferation. The smaller population size of INF exposed populations is attributable to the reduction in proliferation rate relative to the loss rate. The rates of PWM induced proliferation by T cells and non-T cells were approx equal and reduced similarly in the presence of INF. The observed fraction of the population composed of T cells remained stable up to 144 hr for both cells treated and not treated with INF. The enrichment in the relative number of plasmacyte producing either kappa or lambda light chain antibody, due to PWM induced B cell differentiation, was reduced by INF. The observed reduced protein synthesis rate corroborates the reduced enrichment of immunoglobulin producing cells due to INF. (28 Refs)

8. SELENIUM AND THE ACUTE EFFECTS OF THE CARCINOGENS, 2-ACETYLAMINOFLUORENE AND METHYLAZOXYMETHANOL ACETATE.

Banner WP, Tan QH, Zedeck MS
(c/o Zedeck) Lab. Pharmacology, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
Cancer Res; 42(8):2985-2989 1982

Selenium inhibits the development of 2-acetylaminofluorene-induced hepatic tumors and methylazoxymethanol-induced colon tumors. It has been suggested that selenium exerts these protective effects by inhibiting metabolic activation of the carcinogen. We have studied the effects of selenium upon the acute inhibition of RNA and DNA synthesis induced by 2-acetylaminofluorene or methylazoxymethanol in intact liver, regenerating liver, and colon of weanling male Sprague-Dawley rats. Some animals received selenium in the drinking water (4 ppm) for 1 wk, while others received a single injection of selenium (1 mg/kg ip) prior to being treated with the carcinogens. No protection against the effects of the carcinogens on RNA or DNA synthesis was noted with either treatment of selenium. Disulfiram did protect against the 2-acetylaminofluorene-induced inhibition of hepatic RNA synthesis, and pyrazole prevented the inhibition of RNA synthesis induced by methylazoxymethanol in both liver and colon. Serum selenium levels are reported. The data indicate that selenium does not influence the acute alterations induced by the carcinogens 2-acetylaminofluorene or methylazoxymethanol and suggest that the tumor-preventive effects of selenium are probably due to a mechanism other than interference with carcinogen activation and interaction with cellular macromolecules. (Author abstract) (34 Refs)

9. EFFECTS OF PHORBOL MYRISTATE ACETATE AND A LYMPHOKINE ON CYCLIC 3':5'-GUANOSINE MONOPHOSPHATE LEVELS AND PROLIFERATION OF MACROPHAGES.

Hadden EM, Sadlik JR, Coffey RG, Hadden JW
Dept. Pharmacology, Univ. South Florida Medical Center, Tampa, FL, 66312
Cancer Res; 42(8):3064-3069 1982

The tumor promoter phorbol myristate acetate (PMA) was compared to a lymphokine macrophage mitogenic factor (MMF) for its ability to induce replication of guinea pig peritoneal and alveolar macrophages. Like MMF, PMA induces DNA synthesis of both cell populations with peak thymidine incorporation at 72 hr of culture. Optimal concentrations of PMA for the peritoneal and alveolar cells were 1.6 x 10(-7) and 1.6 x 10(-9) M, respectively. The magnitude of the effect is slightly less than MMF but greater than that of phytohemagglutinin or concanavalin A. Indomethacin added to inhibit prostaglandin synthesis potentiates the effects of MMF but has little effect on the actions of PMA and the other mitogens. Potentiation by indomethacin of the effects of PMA on the peritoneal cell was observed only at the suboptimal concentration of PMA (1.6 x 10(-8) M). By adherence criteria and density gradient fractionation, the cell responding to PMA is confirmed to be the macrophage. Cell counts and nuclear radioautography confirm that replication in this system is reasonably well reflected by thymidine incorporation. The effects of PMA and its analogs as macrophage mitogens correlate with their tumor-promoting effects. Both PMA and MMF induce early increases in peritoneal macrophage levels of cyclic 3':5'-guanosine monophosphate without changes in the levels of cyclic 3':5'-adenosine monophosphate. These studies indicate that PMA offers a

useful probe of macrophage function. (Author abstract) (41 Refs)

10. 3T3 FIBROBLASTS ARE STIMULATED BY 12-O-TETRADECANOYL-PHORBOL-13-ACETATE TO PRODUCE THYMOCYTE-ACTIVATING FACTORS.

Okai Y, Tashiro H, Yamashita U
Dept. Immunology, Univ. Occupational and Environmental Health, Sch. Medicine, Yawatanishi-ku, Kitakyushu 807, Japan
FEBS Lett; 142(1):93-95 1982

The supernatant of cultures in which 3T3 fibroblasts were grown in the presence of 12-O-tetradecanoyl-phorbol-13-acetate was shown to contain several factors which regulate thymocyte DNA synthesis. Sephadex G-100 column chromatography revealed activity in two fractions. Biochemical study indicated the factors are proteins and that their properties are considerably different from one another. (8 Refs)

11. COMPARATIVE TUMOR INITIATING ACTIVITIES OF N-NITROSOMETHYLBENZYLAMINE AND N-NITROSODIMETHYLAMINE IN RAT LIVER.

Labuc GE, Archer MC
(c/o Archer) Dept. Medical Biophysics, Univ. Toronto, Ontario Cancer Inst., 500 Sherbourne St., Toronto M4X 1K9, Canada
Carcinogenesis; 3(5):519-523 1982

The tumor initiating activities of nitrosomethylbenzylamine (NMBzA), an esophageal carcinogen, and nitrosodimethylamine (NDMA), a hepatocarcinogen, were compared in rat liver using modifications of the initiation assays of Tsuda et al and Pitot et al. Equimolar doses of NMBzA or NDMA (33.5 umol/kg) were injected ip into male Sprague-Dawley rats 18 hr after partial hepatectomy. Following selection, foci of preneoplastic hepatocytes were detected by histochemical staining for gamma-glutamyltranspeptidase. Nitrosomethylamylamine (NMAmA, 115 umol/kg), also an esophageal carcinogen, was tested in the assay of Tsuda et al, and nitrosodimethylamine (NDEA, 33.5 umol/kg), a hepatic and esophageal carcinogen, was tested in the assay of Pitot et al. Both NDMA and NDEA induced significant increases in the number of preneoplastic foci above background. In contrast, neither NMBzA nor NMAmA increased the number of foci above background. Microsomes from regenerating liver had a lower capacity to metabolize both NDMA and NMBzA compared to microsomes from intact liver, but the decrease in activity was similar for both compounds. Neither NDMA nor NMBzA significantly inhibited the first wave of DNA synthesis in vivo in the regenerating liver. The results demonstrate that in contrast to NDMA and NDEA, NMBzA and NMAmA lack tumor initiating activity in the liver. (Author abstract) (37 Refs)

12. BOTH TUMOR PROMOTERS AND PROSTAGLANDINS STIMULATE DNA SYNTHESIS IN PRIMARY HEPATOCYTES INCUBATED IN CALCIUM-DEFICIENT MEDIUM (MEETING ABSTRACT).

Romano F, Armato U
Department of Human Anatomy, University of Padua, I-35100 Padua, Padua, Italy
In Vitro; 18(3,part2):302 1982

Reportedly, prostaglandin (PG) synthesis is elevated in cells exposed to tumor promoters, some of which PGs may even structurally resemble. In an investigation of the role of PGs in the promotion of an initiated liver neoplastic process we used primary cultures in neonatal rat hepatocytes grown in a medium containing either a normal or a very low calcium (Ca) complement. Thus, within a wide concentration range two established promoters, namely TPA (12-O-tetradecanoyl-phorbol-13-acetate) and phenobarbital, and the presumptive one melittin both in normal and low Ca medium were equally capable of nearly doubling the quota of 4-day-old hepatocytes flowing into DNA synthesis within the first 24 hours of treatment. Likewise, three PGs, namely A1, E1 and E2, administered at 10(-9) M enlarged by a factor of 1.8 the size of the 24-h fraction of DNA-synthesizing hepatocytes irrespectively of their incubation either in normal or low extracellular Ca. These results show that tumor promoters can promptly disconnect DNA synthesis from the physiological Ca-dependent control system. (1 Ref)

13. PERTURBATION OF CELL PROLIFERATION IN VIVO BY MISONIDAZOLE (MEETING ABSTRACT).

Rockwell S, Irvin CC, Graf CH
Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT, 06510
Program and Abstracts of the 6th Annual Meeting of the Cell Kinetics Society, March 19-21, 1982, Houston, Texas. The Cell Kinetics Society 38 pp., 1982.

Misonidazole, a hypoxic cell radiosensitizer, was found to perturb DNA synthesis patterns in mouse tumors and normal tissues in vivo. Tritiated thymidine uptake in the tissues (spleen, bone marrow, gut, liver, skin, and solid EMT6 tumors) was estimated at different times after treatment of the mice with 1 mg/gm misonidazole, using scintillation counts of the tritium content in disrupted tissue samples to estimate thymidine uptake. Uptake was depressed for several hours after misonidazole treatment in spleen, bone marrow, gut, and tumors. Such perturbations could result from direct effects of misonidazole on DNA synthesis, as have been reported for aerobic cells in vitro, or might reflect proliferative changes produced indirectly, because of the physiologic effects of the drug on the mouse. Proliferative changes induced in cell cultures, tumors, and normal tissue should be considered when interpreting radiobiology studies using radiosensitizers. (no Refs)

14. CORRELATION BETWEEN CHEMICAL STRUCTURE OF AMINO-SUBSTITUTED ANTHRAQUINONES AND IN VITRO BIOLOGICAL ACTIVITY (MEETING ABSTRACT).

Kimler BF, Czapansky K, Cheng CC
Department of Radiation Therapy, University of Kansas Medical Center, Kansas City, KS, 66103
Program and Abstracts of the 6th Annual Meeting of the Cell Kinetics Society, March 19-21, 1982, Houston, Texas. The Cell Kinetics Society 38 pp., 1982.

A consistent correlation has been established between the presence of a particular substituent aminoalkylamino side chain and the concentration of various anthraquinone compounds required to produce a certain level of cell kill in cultured Chinese hamster cells. In general, the same correlation holds for the biological endpoints of blockade of cell cycle progression, inhibition of DNA and RNA synthesis, and inhibition of exponential cell growth. However, in each series there are exceptions, ie, the concentration required to produce a certain level of effect is different than predicted from a linear correlation. This suggests differences in the mechanisms by which the particular perturbations of cellular metabolism are produced; and may be helpful in the elucidation of the site of action of substitute anthraquinones on mammalian cells. (no Refs)

15. PROLIFERATIVE RESPONSES OF HUMAN PROSTATIC NEOPLASIA AND RAT PROSTATE IN ORGAN CULTURE (MEETING ABSTRACT)

Riches AC, Mistry L, Buchanan L, Dattani G, Weaver JP

Department of Anatomy and Experimental Pathology, University of St. Andrews, St. Andrews, Scotland

Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh Scotland. British Association for Cancer Research 78 pp., 1982.

In an attempt to provide a model for investigating hormone responsiveness and sensitivity to chemotherapy of prostatic neoplasia, the proliferative responses of human and rat prostate have been followed in organ culture using 125I-iododeoxuridine uptake (125IUdR) to monitor DNA synthesis. In serum free cultures, testosterone induced a marked increase in DNA synthesis in prostates from 4-6 month old rats, reaching maximal levels after 4 days in culture, after which the uptake decreases to control unstimulated levels. This proliferative response is dose dependent and is maximal at testosterone concentrations of 4 x 10-9M to 4 x 10-6M, whereas in prostates from 12 month old rats the response was less marked. Human benign prostatic hyperplasia showed a minimal response to testosterone stimulation, a similar pattern to that seen in the older rats. The proliferative response of human benign prostatic hyperplasia increases up to day 3 to 4 in culture and then declines in both control and hormone treated groups and may represent repair processes which appear to be hormone dependent. Explants from prostatic carcinoma were well maintained in organ culture but had to be carefully selected using frozen sections to obtain cellular areas in the transurethrally resected tissue samples. No marked changes in the histological appearance of the prostatic carcinoma explants were observed in the presence of testosterone, Estracyt or estradiol. Testosterone exhibited some stimulatory effects in 125IUdR uptake whereas both Estracyt (estramustine phosphate) and estradiol were weakly inhibitory. (no Refs)

16. EFFECTS OF DNA SYNTHESIS INHIBITORS ON THE INDUCTION OF HCG SUBUNITS AND ALKALINE PHOSPHATASE IN HELA S3 CELLS.

Park KC

Iowa State University, Iowa City, IA

Diss Abstr Int [B]; 42(11):4396-B 1982

The synthesis of human chorionic gonadotropin (hCG) alpha- and beta-subunits and alkaline phosphatase has been examined in HeLa S3 cultures. In general, compounds which influenced deoxynucleotide metabolism were good inducers of these ectopic proteins, while agents which altered DNA structure by intercalation or covalent binding were poor inducers. The greatest induction was achieved with sodium butyrate. Induction of ectopic products was synergistic (or at least additive) when intermediate inducers were added in combination with butyrate, suggesting different modes of action for these compounds. The simultaneous addition to cultures of various paired combinations of these intermediate inducers resulted in no greater induction than that observed with either drug alone, indicating similar or competing mechanisms among them. Addition of RNA or protein synthesis inhibitors to cell culture medium containing sodium butyrate, aphidicolin, or phosphonoformic acid abruptly ceased the production of hCG-alpha subunit indicating RNA and protein synthesis were both required for induction of this protein. (no Refs)

17. DNA METABOLISM IN ISOLATED FIBROSARCOMA SUBPOPULATIONS (MEETING ABSTRACT).

Brock WA, Swartzendruber DE, Grdina DJ

M.D. Anderson Hospital and Tumor Institute, Houston, TX, 77030

Program and Abstracts of the 6th Annual Meeting of the Cell Kinetics Society, March 19-21, 1982, Houston, Texas. The Cell Kinetics Society 38 pp., 1982.

Density separated murine fibrosarcoma (FSa) tumor subpopulations were characterized by measuring in vivo and in vitro 3H-thymidine incorporation, endogenous pools of thymidine-triphosphate (TTP), TTP specific activities, and by two parameter flow cytometric analysis. The lighter cells, presumably well oxygenated, have relatively high endogenous levels of TTP, lower TTP specific activities, and higher levels of in vivo 3H-thymidine incorporation than do the more dense ones, which are predominently hypoxic. This suggests that the denser populations are both synthesizing DNA at lower rates and are less able to concentrate 3H-thymidine from the circulation than are the lighter cells. In contrast, all FSa populations labeled in vitro show relative uniform 3H-thymidine incorporation, consistent with the proportion of cells in S-phase as determined by FCM. In addition, FCM shows a relatively higher proportion of quiescent cells in the dense cell populations. These data support the conclusion that the hypoxic cell fraction of FSa tumors is more dense than the well oxygenated cells and that under proper conditions these cells are able to recover and synthesize DNA at normal levels. (no Refs)

18. AUTORADIOGRAPHIC STUDIES ON NUCLEIC ACID SYNTHESIS OF HUMAN GASTRIC CANCER CELLS. I. RELATIONSHIP BETWEEN NUCLEIC ACID SYNTHESIS OF CANCER CELLS AND CLINICOPATHOLOGICAL FINDINGS.

Inoue K

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Nippon Geka Hokan; 51(2):315-326 1982

Surgical specimens were obtained from 22 patients (16 men, 6 women; 32-80 yr old) who had gastric cancer, and the rate of cell proliferation and cell viability of the cells in the central portion of 20/21 tumors were measured with the aid of in vitro 3H-thymidine and 3H-uridine labeling. Thymidine labeling indexes (TLI), uridine labeling indexes (ULI), and mitotic indexes were calculated based on the most active parts of the tumors. The overall TLI of all of the tumors wass 22.4 +-7.9% and the MI was 19.6 +-5.5%. TLI values were decreased in the tumors that were classified as advanced with respect to depth of tumor invasion and stage. No significant differences in TLI were observed between the primary tumor nest and metastatic lymph node tumor nests, av diameter of the primary tumor nest, macroscopic type of the tumors, presence or absence of lymph node metastasis, histological types, and type of infiltrative proliferation. TLI were lower for the scirrhotic type tumor than for the medullary type and were lower for tumors invading the subserosal and tunica muscularis propria layers than for those invading the mucosal layer. The overall ULI of all the tumors was 92.7 +-6.3%. While no consistent correlation was observed between the ULI and pathoclinical findings, RNA synthesis was adequately preserved even in cancer cells with decreased DNA synthesis. (44 Refs) (In JPN)

19. [3H]THYMIDINE LABELS LESS THAN HALF OF THE DNA-SYNTHESIZING CELLS IN THE MOUSE TUMOUR, CARCINOMA NT.

Hamilton E, Dobbin J
Dept. Oncology, Middlesex Hosp. Medical Sch., London W1, England
Cell Tissue Kinet; 15(4):405-411 1982

We have studied carcinoma NT, a transplantable mouse adenocarcinoma of spontaneous origin. Cells labeled with [3H]thymidine ([3H]TdR) were restricted to a narrow zone around the periphery of this tumor and were also found in rings up to 50 um wide, around isolated blood vessels in the central necrotic area. Labeling with [3H]deoxyuridine ([3H]UdR), another DNA synthesis precursor produced a very different pattern. The labeled zone around the periphery was much wider than with [3H]TdR, and [3H]UdR labeled cells were found up to 110 um from isolated vessels. [3H]Iododeoxyuridine ([3H]IUdR) gave the same pattern of labeling as [3H]UdR. In the heavily labeled zone, within 1 mm of the tumor periphery, the labeling index (LI) was 51% after [3H]UdR or [3H]IUdR injection, and only 36% with [3H]TdR. The data show that at least half of the DNA-synthesizing cells in this tumor did not incorporate [3H]TdR. Previous workers reported cell loss factors for carcinoma NT of 60% calculated from [3H]TdR labeling data and 30% from the rate of loss of [125I]UdR. The present work suggests that calculations based on [125I]UdR data are more likely to be accurate for carcinoma NT than those using [3H]TdR data. (Author abstract) (15 Refs)

20. MECHANISMS OF TCGF PRODUCTION BY CLONED T-CELL LINES: CHARACTERIZATION OF PRODUCER LYMPHOMA LINES AND KINETICS OF PRODUCTION.

Smith RT, Shimizu S, Norcross MA, Maino VC
Tumor Biology Unit, Dept. Pathology, Univ. Florida, Coll. Medicine, Gainesville, FL, 32610
Clin Immunol Immunopathol; 23(2):286-296 1982

The activation requirements and kinetics of production of T-cell growth factor are described for a stable, cloned T-lymphoma line, EL4-G-12. The line yields a product which has the biochemical and biological attributes of TCGF of normal cell origin examined thus far. Production is dependent upon a transmembrane signal which can be provided by known T-cell-activating lectins, and by antibody to a specific ligand on the Thy-1 molecule, if accessory cells or factors are provided. Production is rapid, beginning within 4 hr, and ceasing between 12 and 18 hr depending upon cell density, signal intensity, and culture conditions. DNA synthesis ceases as production begins, and release is not a result of cell death. It has not yet been determined whether rapid synthesis de novo, or activation and release of an inactive hormone precursor explains the production kinetics observed. (Author abstract) (17 Refs)

21. SENSITIVITY TO MACROPHAGE-MEDIATED CYTOSTASIS IS CELL CYCLE DEPENDENT.

Hamilton TA, Fishman M, Crawford G, Look AT
Div. Immunology, St. Jude Children's Res. Hosp., Memphis, TN, 38101
Cell Immunol; 69(2):363-373 1982

We have examined the sensitivity of proliferating lymphoid cells in different phases of the cell cycle to macrophage-mediated cytostatic activity. These studies evaluated the ability of target cells enriched in individual cell cycle phases to pass into the next phase during brief (2-6 hr) periods of coculture with activated or nonactivated peritoneal macrophages. Both normal (concanavalin A-stimulated spleen cells) and neoplastic (Gross virus-induced thymic lymphoma) cells were analyzed. Spleen cells or lymphoma cells were first separated by centrifugal elutriation into populations highly enriched for G1, S, or G2/M phases of the cell cycle and cultured in the presence of nonactivated or activated macrophages for periods of 2, 4, or 6 hr. The cellular DNA content of recovered nonadherent target cells was then analyzed by flow cytometry after staining with propidium iodide. Macrophage contamination of target cell populations was insignificant under these conditions. Nonactivated macrophages did not affect target cell cycle traverse when compared with target cells cultured alone. Activated macrophage mediated cytostatic activity resulted in complete block of the transition of cells in G1 phase into S phase and of the further accumulation of DNA by cells in early S phase. Cells already in mid to late S phase were able to continue DNA replication at rates nearly equivalent to control cells. There was no inhibition of the passage of cells through G2 or mitosis. These effects were seen as early as 2 hr of macrophage-target cell coculture and both normal and neoplastic cells exhibited identical patterns of cell cycle phase sensitivity. (Author abstract) (21 Refs)

22. ASPECTS OF THYMIDINE METABOLISM AND FUNCTION IN CULTURED MAMMALIAN CELLS INFECTED WITH HERPES SIMPLEX VIRUS TYPE 1.

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J Gen Virol; 59(part2):223-234 1982

We have investigated aspects of thymidine metabolism and function in cultured mammalian cells infected with herpes simplex virus under conditions in which virus DNA synthesis was either permitted, or was prevented by inhibiting the virus-induced DNA polymerase with phosphonoacetate. In 30 min pulse-labeling experiments the rates of [3H]thymidine transport into cells and its subsequent phosphorylation both reached max values about 6 hr after infection. During the next 17 hr these rates remained relatively constant in the absence of phosphonoacetate but declined to about 50% of the 6 hr value in the presence of this inhibitor. When the radioactive thymidine was present continuously throughout the infection the accumulated intracellular acid-soluble radioactivity reached max values at about 9 hr. In the absence of phosphonoacetate this declined thereafter, but it remained relatively constant when virus DNA synthesis was prevented. This suggests some established equilibrium between the continuing entry of thymidine into cells and its subsequent removal by excretion. Excretion of thymidine-derived radioactivity was initially established by determining the fate of isotopically labeled host cell DNA. During 24 hr of infection about 50% of the host cell DNA was rendered acid-soluble and of this 60 to 70% was excreted from the cells. High pressure liquid chromatographic analysis of the intracellular acid-soluble radioactivity showed only phosphorylated thymidine derivatives, whereas the excreted radioactivity was exclusively in thymidine. Excretion of intracellular radioactive molecules derived directly from [3H]thymidine in the medium was also observed, but only when virus DNA synthesis was inhibited with phosphonoacetate. Simple kinetic studies with the purified virus-induced DNA polymerase showed that a straight line double-reciprocal plot was obtained when dGTP was used as the limiting substrate (Km = 0.8 uM), but that a biphasic plot was obtained when dTTP was limiting. (28 Refs)

23. INHIBITORY EFFECT OF E-5-(2-BROMOVINYL)-1-BETA-D-ARABINOFURANOSYLURACIL ON HERPES SIMPLEX VIRUS REPLICATION AND DNA SYNTHESIS.

Descamps J, Sehgal RK, De Clercq E, Allaudeen HS
Dept. Pharmacology, Yale Univ. Sch. Medicine, New Haven, CT, 06510
J Virol; 43(1):332-336 1982

The effect of E-5-(2-bromovinyl)-1-beta-D-arabinofuranosyluracil (BVaraU) on herpes simplex virus (HSV) replication was examined and compared with that of E-5-(2-bromovinyl)-2'-deoxyuridine (BVdUrd). The 50% inhibitory dose against HSV type 1 (HSV-1) was 0.1 ug/ml compared with 0.008 ug/ml for BVdUrd; the antimetabolic 50% inhibitory dose of BVaraU ranged from 20 to 95 ug/ml. The addition of 50 ug of BVaraU per ml to HSV-1-infected Vero cells decreased the synthesis of viral and cellular DNA by 37 and 28%, respectively. The 5'-triphosphate (BVaraUTP) competed with dTTP in DNA synthesis by the herpes-viral and cellular DNA polymerases; the apparent Ki values of HSV-1 DNA polymerase, DNA polymerase alpha, and DNA polymerase beta were 0.14, 0.32, and 5 uM, respectively. Thus, BVaraU was a less effective antiherpesvirus agent than BVdUrd; unlike BVdUrd, it did not appear to be internally incorporated into replicating DNA in virus-infected cells. (Author abstract) (13 Refs)

24. EFFECTS OF BENZO[A]PYRENE DIOL EPOXIDE ADDUCTS ON DNA SYNTHESIS IN MAMMALIAN CELLS.

Rinaldy A, Lockhart ML, Deutsch JF, Ungers GE, Rosenberg BH
Walker Lab., Sloan-Kettering Inst., Rye, NY, 10580
Mutat Res; 94(2):383-391 1982

Effects of benzo[a]pyrene diol epoxide (BPDE) adducts in DNA were studied in CV1-P cells infected with SV40 virus treated with BPDE in vitro prior to infection. Replication in CV-1 monkey kidney cells of SV40 DNA containing an av of 11 anti-BPDE adducts/molecule were compared to that of similar DNA containing no adducts. The decreased replication of DNA from BPDE-treated virus may have resulted directly from the presence of BPDE adducts in the DNA, or it may have been due to a decrease in cellular uptake or uncoating of the virus, possibly caused by the presence of BPDE adducts in the viral capsid proteins. Although replication of the DNA of the BPDE-treated virus was initially low, in the course of time the ratio of treated tsA to deletion molecules approached that of the mock-treated control. Viruses with 11 adducts/DNA molecule were only approx 12% viable in spite of the fact that most or all of the DNA molecules are ultimately able to replicate. The loss of viability thus suggested either a persistent defect in transcription of the infecting DNA, caused by BPDE adducts that do not prevent replication and/or a high probability of producing defective progeny molecules during repair or bypass replication of parental molecules containing BPDE adducts. The 12% viability indicated an av of approx 2/11 lethal hits. Data for three adducts/molecule indicated that approx 7% of the adducts were lethal. Results implied that a minor adduct might be responsible for the the loss of viability. (19 Refs)

25. STUDIES WITH APHIDICOLIN ON THE FV-1 HOST RESTRICTION OF FRIEND MURINE LEUKEMIA VIRUS.

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Dept. Medicine, Microbiology, Cell Biology, Albert Einstein Coll. Medicine, Bronx, NY, 10461
J Virol; 43(1):182-190 1982

The murine gene Fv-1 exerts a major control over the replication of Friend murine leukemia virus (F-MuLV). An effect of the gene product has been determined to be at the level of accumulation and integration of viral DNA. Aphidicolin, an inhibitor of eucaryotic DNA polymerase alpha, was studied in murine cells infected either permissively or nonpermissively with regard to the Fv-1 genotype. Results indicated that inhibition of DNA polymerase alpha did not affect the accumulation of Form III viral DNA in either permissive or nonpermissive cells. However, the normal accumulation of circular Form I DNA in permissive cells was inhibited. The block in the accumulation of Form I DNA resembled that occurring in some F-MuLV Fv-1-nonpermissive infections. Additionally, aphidicolin treatment resulted in the accumulation of novel low-molecular-weight viral DNA species, normally detectable in a nonpermissive infection of NIH cells with B-tropic F-MuLV. These data suggest that the Fv-1 gene product may interact with host DNA polymerase alpha to prevent viral replication. (Author abstract) (25 Refs)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

26. THE MUTAGENIC EFFECT OF DEOXYNUCLEOTIDE SUBSTRATE IMBALANCES DURING DNA SYNTHESIS WITH MAMMALIAN DNA POLYMERASES.

Kunkel TA, Silber JR, Loeb LA
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Mutat Res; 94(2):413-419 1982

Relation between relative concentrations of incorrect and correct substrates and frequency of mutagenesis by mammalian DNA polymerases was studied in PhiX174 amber mutants. The mutation frequency and the two different loci on a biologically active DNA template was significantly affected by the ratio of incorrect to correct nucleotides during in vitro polymerization catalyzed by either DNA polymerase-alpha or DNA polymerase-beta. The results provide support for the hypothesis that altered nucleotide pools cause mutations in mammalian cells by decreasing the fidelity of DNA synthesis. (22 Refs)

27. THE ROLE OF ACID AND ALKALINE DNASES AS TUMOUR MARKERS IN CANCER OF THE GENITOURINARY TRACT.

Lykourinas M, Constantinidis C, Spantidos A, Manthopoulos A, Dimopoulos C
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Urol Res; 10(2):67-70 1982

Use of acid DNase and alkaline DNase as tumor markers in cancers of the genitourinary tract was investigated. Serum enzyme levels were measured in 109 patients with urological cancer (68 transitional cell carcinoma of the bladder, 18 pros-

tatic cancer, 10 testicular tumors, 13 penile tumors), in 30 patients with nonmalignant tumors and inflammatory diseases of the urinary tract (papilloma, hypertrophy of the prostate, urethritis, cystitis, epididymo-orchitis, pyelonephritis), and in 38 healthy blood donors. The upper limit of normal was 400 units/ml for acid DNase and 200 units/ml for alkaline DNase. Increased levels of acid DNase were found in 62% of urological cancer patients; alkaline DNase was elevated in 43%. In patients with inflammatory diseases and nonmalignant tumors, elevated acid DNase was found in 30% and elevated alkaline DNase in 13%. In patients with bladder, prostate, or testicular carcinomas, elevated DNase activity was higher in advanced stages. In those with penile carcinoma, elevations in both DNases were seen more often in patients with low stage disease. Pretreatment levels of acid DNase in patients with penile carcinoma were elevated in 43%, during treatment in 49%, and posttreatment in 34%. Thus, estimation of DNase can be used as a cancer marker after careful exclusion of other conditions for monitoring the progress of individual patients. (6 Refs)

MECHANISMS OF DNA REPAIR

28. MECHANISMS FOR CHROMOSOMAL ABERRATIONS IN MAMMALIAN CELLS.

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Mutat Res; 95(1):1-6 1982

Lesions responsible for chromosomal aberrations (CA) induced by ionizing radiations, role of base damage in x-ray-induced CA, and experiments involving repair inhibitors are discussed. The frequencies of DNA-double strand breaks induced by x-rays alone, as well as in combination with Neurospora crassa endonuclease (NE) were measured by neutral sucrose-gradient centrifugation analysis to confirm the cytological findings and their interpretation. X-irradiation of CHO cells with 30 kilorad led to 8.8 double-strand breaks/10(10) dalton of DNA, and post treatment of the cells with NE led to a doubling of the frequencies of double-strand breaks induced by x-rays supporting the conclusion that DNA double-strand breaks are the most probable lesions that lead to the formation of CA induced by ionizing radiations. When G0 or G1 cells from patients with ataxia telangiectasia were irradiated with x-rays, both chromosome and chromatid types of aberration were obtained in contrast to mainly chromosome-type aberrations obtained in normal cells. Several inhibitors of DNA synthesis, such as cytosine arabinoside, aphidicolin, and hydroxyurea, as well as inhibitors of poly(ADP-ribose) polymerase (PRP), have been used in x-ray experiments involving human peripheral blood lymphocytes. When human peripheral blood lymphocytes were irradiated and post treated with inhibitors of PRP, such as 3-aminobenzamide, for at least 6 hr prior to stimulation, a doubling of the frequency of aberrations was observed. The results supported the conclusion that DNA double-strand breaks are the most important lesions responsible for radiation-induced CA. (19 Refs)

29. REPAIR OF DNA ALKYLATION INDUCED IN L1210 LEUKEMIA AND MURINE BONE MARROW BY THREE CHLOROETHYLNITROSOUREAS.

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Cancer Res; 42(7):2605-2608 1982

The removal of DNA adducts is an essential step of DNA repair following exposure to chloroethylnitrosoureas. Adduct removal was evaluated in both L1210 and murine bone marrow DNA for lesions induced by three chloroethylnitrosoureas. 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea, a marrow-toxic agent with high carbamoylating activity, was not removed in either system for at least 6 to 12 hr. These results were compared with those obtained with two glucose-linked chloroethylnitrosoureas, chlorozotocin and 1-(2-chloroethyl)-3-(beta-D-glucopyranosyl)-1 nitrosourea. Both of these agents have low marrow toxicity at therapeutic doses. Chlorozotocin, which has very low chemical carbamoylating activity, was found to permit approx 40% removal of drug-derived DNA adducts in both systems within the first 6 hr and approx 50% by 18 hr. The second glucose-linked analog, 1-(2-chloroethyl)-3-(beta-D-glucopyranosyl)-1 nitrosourea, has relatively high carbamoylating activity and was found to inhibit early removal of DNA adducts as effectively as does 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. It would thus appear that the selective marrow-sparing property of the sugar-linked chloroethylnitrosoureas is not dependent upon carbamoylation-mediated differences in the rate and extent of DNA adduct removal. In view of the comparable therapeutic activity of the three drugs for L1210 leukemia, therapeutic efficacy does not appear to be impaired by the increased rate of adduct removal observed with chlorozotocin in this system. (Author abstract) (18 Refs)

30. REACTION OF ANTHRAMYCIN WITH DNA. BIOLOGICAL CONSEQUENCES OF DNA DAMAGE IN NORMAL AND XERODERMA PIGMENTOSUM CELL LINES.

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J Biol Chem; 257(11):6207-6215 1982

Anthramycin, an antitumor antibiotic produced by Streptomyces refuineus, produces a well defined covalent adduct with DNA and lies within the narrow groove of DNA, attached through a thermal-labile covalent amino linkage to the exocyclic amino group of guanine, without detectable distortion of the helix. This paper described results in which the biological consequences of DNA damage and repair by repair-proficient and a repair-deficient xeroderma pigmentosum (XP 12RO) cell line are presented. Anthramycin has been shown to produce excision-dependent single and double strand breaks in DNA, both of which appear to persist many hours after removal of the drug from the media. The lower ability of the xeroderma pigmentosum cell line to remove anthramycin lesions from DNA is correlated with a decreased cell survival. The biological consequences of DNA damage (genetic effects, DNA strand breakage, and cytotoxicity) are discussed with respect to the defined structure and stability of the anthramycin-deoxyguanosine adduct. (Author abstract) (31 Refs)

31. REPAIR OF TRANS-PT(II)DIAMMINEDICHLORIDE DNA-PROTEIN CROSSLINKS IN NORMAL AND EXCISION-DEFICIENT HUMAN CELLS.

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Lab. Pathology, NCI, NIH, Bethesda, MD, 20205
Mutat Res; 94(2):277-284 1982

Sensitivity of xeroderma pigmentosum (XP) group A fibroblasts to trans Pt(II)-diamminedichloride (trans-Pt) was studied to find out whether the repair of DNA-protein crosslinks (DPC) induced by trans-Pt was deficient in XP cells. DPC, produced in cells by trans-Pt, was measured by the alkaline elution technique. When the cells were treated with trans-Pt and exposed to 3 kilorads, the level of cross linking was very similar in both normal and XP cells. The level of DPC increased somewhat in both cell types in the first several hours after trans-Pt was removed. By 12 hr, the DPC level was significantly less in normal as compared to XP cells. By the 18 hr the DPC level was approx half the maximal in the normal cells but decreased little in XP cells. Trans-Pt alone induced a negligible level of DNA single strand breaks (SSB) in both cell types; cytosine arabinoside and hydroxyurea induced a small but consistent increase in elution. With the polymerase inhibitor and trans-Pt, a marked increase in elution was observed in normal but not XP cells. In order to determine if trans-Pt was more toxic to XP cells, colony survival experiments were preformed. Trans-Pt was more toxic to XP cells; a concentration of trans-Pt 3-4x greater was needed in normal cells to produce the same toxicity observed in XP cells. A similar result was also observed with CRL1187 cells. (17 Refs)

32. DNA-REPAIR DEFICIENCIES DO NOT AFFECT INTERCALATOR-INDUCED CYTOTOXICITY OR DNA SCISSION IN HUMAN CELLS.

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Building 37, Room 5D17, 9000 Rockville Pike, Bethesda, MD, 20205
Mutat Res; 104(4/5):295-304 1982

Toxicity of the DNA intercalator 4'-(acridinylamino)-methanesulfon-m-aniside (m-AMSA) was compared to the toxicity of xeroderma pigmentosum (XP) and ataxia telangiectasia (A-T) cells. Normal human fibroblasts and the protein associated DNA-strand breaks produced by m-AMSA are also discussed. The efficiency of m-AMSA in reducing colony-forming ability was the same in all three human fibroblast lines. Quantification of m-AMSA production of DNA-strand breaks by alkaline elution also revealed no differences among the three lines. Results differed from those of experiments which measured cytotoxic effect and DNA-strand breaks produced by damaging agents. Scission produced by m-AMSA also disappeared similarly in normal, XP, and A-T lines. The m-AMSA breaks in all three cell lines were associated with comparable magnitudes of DNA-protein crosslinking. The concentration of m-AMSA associated with each cell type was measured by the use of radioactive compound. The normal cells displayed no reduction in either m-AMSA sensitivity or single-strand break production. A true increased sensitivity of the variant cells thus was not masked by deficient m-AMSA uptake. The effects upon DNA biochemistry and cell survival produced by m-AMSA appeared to be mediated by cellular factors other than those that are deficient in human cell lines abnormally sensitive to other DNA-reactive agents. (31 Refs)

33. RELATIONSHIP BETWEEN EXCISION REPAIR AND THE CYTOTOXIC AND MUTAGENIC EFFECT OF THE 'ANTI' 7,8-DIOL-9,10-EPOXIDE OF BENZO[A]PYRENE IN HUMAN CELLS.

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Mutat Res; 94(2):435-447 1982

Effects of the anti isomer of 7,8-diol-9,10-epoxide of benzo[a]pyrene (anti BPDE) were studied in xeroderma pigmentosum XP12BE and normally excising diploid human cells (NF). To determine if the time available for excision repair prior to onset of semi-conservative DNA replication plays a significant role in decreasing the potentially mutagenic effect of anti BPDE, NF cells were synchronized by density inhibition, released, and treated at expression densities from a common stock soln of tritiated anti BPDE approx 16 or 1 hr prior to onset of S. The NF cells treated in situ just prior to S exhibited the highest frequency of induced mutations. The frequencies observed in NF cells treated in situ in early G1 approx 16 hr prior to S or treated in density-inhibited G0 state were significantly (12-to 16-fold) lower than those of NF cells treated just prior to S. No such difference was observed in the excision repair-deficient XP12BE cells. Results supported the hypothesis that the number of DNA lesions remaining unexcised during S determines the mutagenic effect of the agent. Results indicated that DNA replication occurred on templates containing unexcised residues and that the number of anti BPDE adducts remaining unexcised was approx the same in the replicated and the non-replicated fraction of parental DNA. At the onset of S, the number remaining bound in NF cells was approx 2.5 residues/10(6) nucleotides. (14 Refs)

34. RESTITUTION OF CHROMATID AND ISOCHROMATID BREAKS INDUCED IN THE G2 PHASE BY ACTINOMYCIN D.

Cherry LM, Hsu TC
Dept. Cell Biology, Univ. Texas M. D. Anderson Hosp. and Tumor Inst., Houston, TX, 77030
Environ Mutagen; 4(3):259-265 1982

Lymphocytes treated with actinomycin D (AD; 3.5 ug/ml) for 90 min showed single chromatid breaks, a high frequency of chromatid exchange figures (translocations, inversions, rings) and closed isochromatid breaks. These changes indicated that a type of DNA repair leading to chromosome aberrations had occurred prior to cell fixation. At least some of the breaks that occur after AD treatment in G2 represent true DNA damage and were not merely artifacts of fixation. (8 Refs)

35. IMPAIRED REPAIR CAPACITY OF DNA STRAND BREAKS INDUCED BY 125I-TRIIODOTHYRONINE IN CHINESE HAMSTER CELLS.

Sundell-Bergman S, Johanson KJ
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Biochem Biophys Res Commun; 106(2):546-552 1982

The induction of DNA strand breaks in Chinese hamster cells following (125I)-triiodothyronine (T3) labelling was investigated using the sensitive DNA unwinding technique. While nonradioactive T3 did not produce strand breaks, the 125iodinated form did. Effects of T3 were similar in V79, HeLa and GH1 cells. The inefficiency of DNA repair observed even after low 125I doses (0.4 dpm per cell) suggested that double strand breaks or more serious DNA damage had occurred. Re-

sults indicated that the 125I-decay products were within 60 nanometer from DNA, a distance at which 125I decay yields a high LET-type radiation. A comparison with previous work indicated that damage to DNA was more severe after (125I)-T3 than after incorporation of 125I directly into DNA. Possible reasons from this are discussed. These results suggest that the thyroid may not necessarily be the target organ for iodine isotope-induced radiation damage. Since a very high number of T3 receptors occur in the anterior pituitary, especially during growth, use of isotopes of this compound in children could result in disturbed hormone function. Another implication is the possible use of (125I)-T3 in therapy of tumors with a high number of nuclear receptors for T3. (17 Refs)

36. STUDY OF MOLECULAR MECHANISMS OF RADIATION DEATH OF LYMPHOID CELLS. COMMUNICATION 3. REPAIR OF SUPERHELICAL DNA FROM THYMOCYTE SUBPOPULATIONS WITH DIFFERENT RADIOSENSITIVITY.

Filippovich IV, Soldatenkov VA, Sorokina NI, Romantsev EF
No affiliation given
Radiobiologiia; 22(2):165-170 1982

The rate of repair of superhelical DNA was studied in large (radioresistant) and small (radiosensitive) thymocytes from male (CBA x C57BL)F1 mice. Isolated thymocytes were irradiated with 250 rads. The large thymocytes showed more rapid reunion and incision than the small thymocytes. The relaxation of superhelical DNA in large thymocytes was similar to that in small thymocytes. It was concluded that the differences in the rate of DNA repair can not be explained in terms of different density of superhelical DNA. (18 Refs) (In RUS)

37. REPAIR OF DOUBLE STRAND BREAKS IN X-IRRADIATED EHRLICH ASCITES TUMOUR CELLS AND THE EFFECTS OF THE DNA INHIBITOR BETA-ARA A (MEETING ABSTRACT)

Bryant PE, Blocher D
GSF-Abteilung fur Biophysikalische Strahlenforschung, 20 Paul Ehrlich Strasse, 6000 Frankfurt/M, W. Germany
Molecular and Cellular Mechanisms of Irradiation, March 17-19, 1982, Neuherburg, W. Germany. Gesellschaft fur Strahlen und Umweltforschung MBH 65 pp., 1982.

The repair of DNA dsb has been followed in cells after x-irradiation using the unwinding and the neutral sucrose centrifugation methods. Dsb, induced with a frequency of 40 dsb/cell/Gy, repaired with a time constant t37 equals 2-4h. Beta-ara A strongly inhibited dsb repair; the inhibition was complete at about C equals 400 umol/l. Experiments designed to simulate the procedure used in plating of cells to measure survival, showed that when Beta-ara A was washed away from cells after treatment, the repair of dsb returned. Beta-ara A has been shown to remove the shoulder from the survival curve of this cell line (Iliakis 1980), therefore it was of interest to measure the breaks remaining at longer times following irradiation. Although repair of dsb returned after washing away the drug, it was found that more breaks persisted in Beta-ara A treated cells than in the control untreated cells. A larger number of dsb were also measured at times corresponding to the time of first cell division, in lightly irradiated cells. These additional dsb may contribute to the increased yield of chromosome aberrations observed, after irradiation and Beta-ara A treatment, at first mitosis which probably cause the increased cell killing observed. It is postulated however that an increased level of misjoining of DNA strands also contributes to the increased aberration formation seen after Beta-ara A treatment of irradiated cells. (2 Refs)

38. RADIATION-INDUCED THYMINE BASE DAMAGE IN REPLICATING CHROMATIN.

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Dept. Radiology, Univ. Utah Medical Center, Salt Lake City, UT, 84132
Radiat Res; 90(3):564-574 1982

When HeLa cells labeled with (3H)thymidine in their parental or mature chromatin DNA or their nascent DNA were exposed to increasing doses of gamma radiation (50-150 kilorad) a greater damage efficiency was observed in nascent chromatin DNA. Elevated thymine damage efficiency in replicating DNA was observed in both irradiated whole cells and isolated nuclei. Both the enzyme digestion (micrococcal nuclease) kinetics of DNA in mature and nascent chromatin, as well as the limit of digestion, were the same in control and irradiated cells. The size of the DNA fragments produced during enzyme digestion and the distribution of the various fragment sizes at equal digestion levels were the same in both control and irradiated cells. The rate at which the excess sensitivity of nascent chromatin DNA decreased to mature chromatin DNA levels was similar to the rate at which nascent chromatin matures, a half-time of slightly more than 2 min in both cases. Hyperthermic exposure reduced the sensitivity of nascent chromatin to parental levels. Deproteinized small fragments of nascent DNA and large fragments of mature or parental DNA were equally sensitive to thymine damage production. When nuclei were exposed to 150 kilorad of gamma radiation and then digested with micrococcal nuclease to a level which solubilizes mainly spacer DNA, the frequency of 5',6'-dihydroxydihydrothymine (tgamma)-type thymine damage in the acid insoluble DNA (principally nucleosome core DNA) was similar to that in whole, undigested chromatin DNA. These results indicate that gamma-type thymine base damage is uniformly distributed across nucleosome core and spacer DNA. (15 Refs)

39. CRITICAL PERIODS DURING THE IN SITU REPAIR OF RADIATION-INDUCED DNA DAMAGE IN RAT CEREBELLAR NEURONS AND 9L BRAIN TUMOR CELLS.

Wierowski JV, Thomas RR, Ritter P, Wheeler KT
Div. Radiation Oncology, Univ. Rochester Cancer Center, Box 704, 601 Elmwood Ave., Rochester, NY, 14642
Radiat Res; 90(3):479-488 1982

The existence of critical periods during repair of radiation-induced DNA damage such that a second dose delivered during these periods causes an accumulation of DNA damage was investigated. DNA repair kinetics for cerebellar neurons and intracerebral 9L tumor cells from the same rat were assayed following the second of two 1,250 rad doses of 137Cs gamma rays separated by various intervals. At the time of the second 1,250 rad exposure, the amount of DNA damage remaining from the first 1,250 rad exposure was 44 rad equivalents or less in tumor cells, and 184 rad equivalents or less in neurons. Alterations in the neuronal DNA repair kinetics observed in the 15 min split-dose experiment were found to be specifically related to the events that occurred or were occurring at the time the second dose was delivered. In neuronal DNA profiles exhibiting an increased amount of DNA sedimenting greater than 250 S in the 15-min split dose experiments, the amount of DNA sedimenting greater than 300 S was also found to be significantly greater than that from unirradiated controls. The physiological consequences of the apparent accumulation of DNA damage observed in split-dose experiments are unknown. For intervals greater than or equal to 15 min between the two 1,250 rad exposures, no critical pe-

riods were found in intracerebral 9L tumor cells. An interval of 15 min to 1 hr between doses appeared to be a critical period for cerebellar neurons, resulting in an increase in fast sedimenting DNA. Molecular events leading to this increase in fast-sedimenting DNA after the second dose remain unknown. Application of this work to clinical radiotherapy is discussed. (15 Refs)

40. INTERCELLULAR CO-OPERATION IN REPAIRING RADIATION-INDUCED SINGLE-STRAND DNA BREAKS.

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Dept. Tumor Biology II, Karolinska Inst. Medical Sch., 104 01 Stockholm, Sweden
Int J Radiat Biol; 41(5):589-593 1982

The role of glutathione (GT) in the contact effect, the enhanced rejoining of single-strand DNA breaks (ssb) in cells irradiated while in close contact, was examined using an experimental system with GT-deficient (GSH-) and GT-proficient (GSH+) human sc fibroblasts. Rejoining of ssb in GSH- cells proceeded at a decreased rate; not more than approx 70% of the radiation-induced breaks were repaired within 1 hr when incubated either in buffer solution or in complete medium. Post-irradiation treatment of GSH- cells with GT increased the rejoining capacity of GSH- cells to normal levels when added immediately after irradiation. Admixture of GSH+ cells to irradiated GSH- cells in a proportion 2:1 also enhanced ssb rejoining capacity. Heavily irradiated GSH+ cells appeared to have a slightly greater effect in enhancing ssb repair than unirradiated cells. Mixing irradiated and unirradiated GSH- cells in a proportion of 1:2 does not appreciably influence the rate of ssb rejoining. When the number of unirradiated GSH- cells in the cell mixture was 15x larger than the number of irradiated GSH- cells, ssb rejoining was enhanced, but remained below the normal level after 1 hr of incubation. Medium conditioned with GSH+ cells slightly enhanced rejoining, while conditioning with GSH- cells was slightly inhibitory. The results indicated that some part of the cellular rejoining process of ssb requires the presence of GT or some other specific aminothiol. (19 Refs)

41. SURVIVAL OF 60CO-IRRADIATED HERPES SIMPLEX VIRUS IN 15 HUMAN DIPLOID FIBROBLAST CELL STRAINS.

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Mutat Res; 94(2):245-255 1982

Survival of gamma-irradiated herpes simplex virus (HSV) was investigated in cells from patients with ataxia telangiectasia (AT), hereditary retinoblastoma, Gardner's syndrome, Fanconi's anemia, and xeroderma pigmentosum (XP). Survival of 60Co-irradiated HSV in normal cells at -75 C was much greater than that of virus irradiated at 4 C. Under the more resistant conditions, approx a 2.5-fold greater dose was required to reduce survival to 1%. Survival of 60Co-irradiated HSV in cell strains derived from diseases associated with DNA repair deficiencies at -75 C was greatly increased if compared with that obtained for repair at the higher temperature (4 C). Survival generally declined more rapidly at lower doses. Only in two cell strains did the survival of HSV differ significantly from that in normal controls. The D0 values (the dose necessary to reduce survival to 37% on the linear portion of the survival curve) for the complementation group A XP strain XP12BE were approx 20% lower than those of controls, regardless of the irradiation conditions. Fanconi's anemia strain was the only cell which exhibited a significantly higher level of virus survival. No significant changes in plaque-forming ability of unirradiated and irradiated (300 kilorad) virus were observed when treatment occurred immediately prior to infection. (39 Refs)

42. INCREASED RATE OF REPAIR OF ULTRAVIOLET-INDUCED DNA STRAND BREAKS IN MITOGEN STIMULATED LYMPHOCYTES.

Hamlet SM, Lavin MF, Jennings PA
Dept. Biochemistry, Veterinary Pathology, and Public Health, Univ. Queensland, Brisbane, 4067, Queensland, Australia
Int J Radiat Biol; 41(5):483-491 1982

Previous results from this laboratory have shown that phytohemagglutinin-stimulated bovine lymphocytes exhibit a peak of ultraviolet-induced DNA repair synthesis 3 to 4 days after addition of mitogen. The level of repair synthesis was approx 10 fold higher than that in unstimulated lymphocytes. We have extended these studies to examine the rate of repair of strand breaks in UV-irradiated bovine lymphocytes. The extent of breakage of DNA was shown to be the same in mitogen-stimulated and unstimulated lymphocytes from two breeds of cattle, when determined by sedimentation of nucleoids on sucrose gradients. However, in mitogen-stimulated cells the time taken to repair DNA strand breaks was 6 hours compared to 12 hours in stationary phase lymphocytes after a UV dose of 5 J/m2. These results suggest that the increased rate of repair of strand breaks is due to the induction of enzymes involved at the post-incision stage of DNA repair. Thus the increased level of repair synthesis observed in earlier work correlates with an increased rate of repair of DNA strand breaks in phytohemagglutinin-stimulated bovine lymphocytes. (Author abstract) (20 Refs)

43. ULTRAVIOLET RADIATION SENSITIVITY OF PROLIFERATING AND DIFFERENTIATED HUMAN NEUROBLASTOMA CELLS.

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Int J Radiat Biol; 41(5):547-556 1982

The effects of ultraviolet (UV) radiation were studied on a cloned line of human neuroblastoma cells in proliferative and differentiated growth modes, the latter being induced by serum deprivation. The neuroblastoma cells were found to be unusually sensitive in comparison with HeLa cells when survival was measured by colony formation in soft agar, the differentiated mode being the most sensitive. Ultraviolet radiation sensitivity was associated with very low DNA repair capacity as measured by DNA repair synthesis and by removal of M luteus endonuclease-sensitive sites from irradiated DNA. The greater sensitivity of the differentiated cells appeared to be related to a greater degree of DNA damage at a given UV dose, resulting from altered cell geometry in the growth mode. The neuroblastoma cells showed little or no post-irradiation inhibition of DNA replication at low UV doses, suggesting that it is the repair process rather than the DNA damage which is responsible for inhibiting replication. (Author abstract) (34 Refs)

44. RECOVERY FROM POST-IRRADIATION INHIBITION OF DNA SYNTHESIS IN AN ULTRAVIOLET-SENSITIVE MUTANT MOUSE CELL.

Hama-Inaba H, Hieda-Shiomi N, Shiomi T, Sato K
Genetics Div., Natl. Inst. Radiological Sciences, 9-1, Anagawa-4, Chiba 260, Japan
Mutat Res; 104(4/5):305-309 1982

Rates of DNA synthesis after irradiation were measured in mouse lymphoma L5178Y cells, UV-sensitive variant Q31, and the hybrid L51.Q1 cells, and affected recovery of UV-inhibited DNA synthesis was observed in mutant cells. Mutant Q31 cells were more sensitive to the lethal effects of UV than were the parental cells and the sensitivity of hybrid L15.Q1 cells was almost equal to that of L5178Y cells. Results indicated that the trait that confers UV sensitivity to Q31 cells is recessive. The effects of UV irradiation were examined in all three cell lines. The rates of DNA synthesis were inhibited in a dose-dependent manner and the degrees of inhibition were approx the same in the three cell lines indicating that UV irradiation inhibited the DNA synthesis at least for the first hr through a common process in mutant, parent, and hybrid cell lines. The change in the rates of DNA synthesis/viable cell was pursued with time of incubation after irradiation. Results obtained with L5178Y cells showed a slight change (105%) in the rate in the sham-irradiated control after incubation. The time course of the rates of DNA synthesis/viable cell was examined in mutant Q31 cells. The sham-irradiated control showed a slight decrease for the first hr, a recovery thereafter, and a constant rate (102%). Results showed that the rates of DNA synthesis in Q31 cells partially recovered after doses of 2.4 and 5 Joule/square meter (J/m2), but the recovery was completely absent after a dose of 10 J/m2. (9 Refs)

45. EXTENT OF EXCISION REPAIR BEFORE DNA SYNTHESIS DETERMINES THE MUTAGENIC BUT NOT THE LETHAL EFFECT OF UV RADIATION.

Konze-Thomas B, Hazard RM, Maher VM, McCormick JJ
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Mutat Res; 94(2):421-434 1982

Predictions that excision repair-proficient cells treated just prior to S will be significantly more sensitive to the cytotoxic and/or mutagenic effect than will be cells exposed many hr prior to S and that cells which are incapable of excision repair will show no difference in sensitivity were tested. Results obtained with UV radiation as the DNA-damaging agent indicated that semi-conservative DNA synthesis is indeed, the 'critical cellular event' responsible for mutation induction. However, the cytotoxic effect of the agent was not significantly increased in normal fibroblasts treated just prior to S phase, even though the cells' excision repair capacity was critical in determining the survival level ultimately attained. (26 Refs)

46. UV-REPAIR IS IMPAIRED IN FIBROBLASTS FROM PATIENTS WITH FANCONI'S ANEMIA.

Schwaiger H, Hirsch-Kauffmann M, Schweiger M
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MGG; 185(3):454-456 1982

Fanconi's anemia, a hereditary autosomal disease with chromosomal instability, elevated incidence of cancer and clinical symptoms is accompanied by a DNA repair deficiency. Fibroblasts from patients with Fanconi's anemia were found to be impaired in the DNA repair of UV damage. Nucleoid decondensation and recondensation after UV irradiation were less efficient in fibroblasts from patients with Fanconi's anemia than in those from a healthy proband. These data confirm our earlier findings that DNA ligase is deficient in Fanconi's anemia. (Author abstract) (12 Refs)

47. DEFECTIVE REMOVAL OF DNA CROSS-LINKS IN A REPAIR-DEFICIENT MUTANT OF CHINESE HAMSTER CELLS.

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Dept. Physics, Univ. Texas System Cancer Center, M. D. Anderson Hosp. and Tumor Inst., Houston, TX, 77030
Cancer Res; 42(8):3106-3110 1982

To further understand the relationships between DNA damage, DNA repair, and cellular end points such as survival and mutation, the repair capacity of a DNA repair-deficient mutant (strain UV-20) of Chinese hamster ovary cells was characterized in response to DNA cross-linking agents. This mutant, previously shown to be hypersensitive to killing by both ultraviolet light and the cross-linking agent mitomycin C, was also found to be extremely sensitive to cis-diamminedichloroplatinum, another DNA cross-linking agent. The efficiency of DNA cross-link removal after treatment with mitomycin C or cis-diamminedichloroplatinum was measured using the technique of alkaline elution and compared in wild-type Chinese hamster ovary cells and strain UV-20. Wild-type cells removed 80 or 95% of the cross-links within 24 hr after treatment with cis-diamminedichloroplatinum or mitomycin C, respectively. In contrast, UV-20 cells, which were equally as susceptible to cross-link damage as were wild-type cells, removed only a small proportion of the cross-links made by either agent. These results emphasize the importance of DNA repair processes in modulating the cytotoxic effects of chemicals that produce DNA cross-link damage and suggest that cross-link repair in Chinese hamster ovary cells is controlled by pathway that also repairs damage from ultraviolet radiation. (Author abstract) (26 Refs)

48. DNA REPAIR SYNTHESIS IN MINIMALLY STRESSED HUMAN LYMPHOCYTES.

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(c/o Norman), Univ. California, Los Angeles, CA, 90024
Int J Radiat Biol; 41(5):535-545 1982

We have estimated the rate of unscheduled DNA synthesis (UDS) in human lymphocytes from measurements of tritiated thymidine incorporation into double-stranded DNA (ds-DNA) during incubation of cells in vitro. Cells were not subjected to stresses except those associated with careful handling, or in certain experiments, mild heating or treatment with phytohemagglutinin (PHA). Contribution of scheduled DNA synthesis (SDS) to incorporation was reduced by inhibiting replication and separating freshly replicated single-stranded DNA from repaired ds-DNA by chromatography. By increasing the incubation temperature, which decreases SDS and increases UDS, the residual contribution of scheduled DNA synthesis to thymidine incorporation into ds-DNA was estimated. Effects of increasing the number of cells in S-phase by phytohemagglutinin were also investigated. Results suggest that: the rate of unscheduled DNA synthesis is about 500 +-100 thymidine molecules incorporated per cell per hr; a temperature-sensitive process, probably hydrolysis of DNA, contributes much of the damage repaired by UDS; background ionizing radiation contributes little to the damage; and damage caused by DNA hydrolysis is repaired much more efficiently than lethal damage caused by

ionizing radiation. Large increases in incorporation into ds-DNA occurred when cells were stimulated with PHA. (Author abstract) (19 Refs)

49. NORMAL SISTER CHROMATID EXCHANGE FREQUENCY IN LONG-TERM SURVIVORS WITH ACUTE LEUKEMIA.

Inoue S, Brown L, Ravindranath Y, Ottenbreit MJ
Children's Hosp. Michigan, Detroit, MI, 48201
Cancer Res; 42(7):2906-2908 1982

We hypothesized that the sister chromatid exchanges assay in acute leukemia long-term survivors may detect: (a) long-term effects of combined chemo- and radiotherapy; and possibly (b) those individuals with inherently deficient DNA repair. Accordingly, we determined the sister chromatid exchanges frequency in 26 blood specimens from 24 acute leukemia long-term survivors (patients) and 14 blood specimens from 13 control subjects (controls). The patients consisted of 23 children with acute lymphocytic and one child with acute myelocytic leukemia. The median length of chemotherapy was 2 years. Eighteen of the 24 patients also received prophylactic fractional central nervous system irradiation for the first 3 years of treatment, and one patient received therapeutic irradiation to the central nervous system. The median off-therapy period at the time of study was 2.5 years with a range of 0 to 7.5 years. The controls consisted of the parents of the patients and laboratory personnel. A mean exchange score per cell was established for each specimen (25 to 30 cells/specimen were scored), and it ranged from 3.0 to 9.7 in the patients and from 3.0 to 11.5 in the controls. A mean +- S.D. calculated from those means was 6.0 +-1.8 for the patients and 6.9 +-2.8 for the controls. They were not significantly different. We conclude that chemo- and radiotherapy produced no persistent DNA alterations detectable by this method. (Author abstract) (20 Refs)

50. PARADOXICAL EFFECT OF 1-BETA-D-ARABINOFURANOSYL CYTOSINE TRIPHOSPHATE ON BLEOMYCIN-INDUCED UNSCHEDULED DNA SYNTHESIS IN PERMEABLE SARCOMA CELLS.

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Experientia; 38(6):657-658 1982

The compound, 1-beta-D-arabinofuranosyl cytosine-5'-triphosphate (araCTP), an inhibitor of DNA synthesis, paradoxically enhanced unscheduled DNA synthesis (USD) induced by bleomycin in permeable mouse sarcoma cells. A greater enhancing effect of araCTP on bleomycin-induced USD was observed with lower concentrations of dCTP in the assay mixture. USD measured without bleomycin in nuclei isolated from mouse sarcoma cells was not enhanced, but inhibited by araCTP. (Author abstract) (15 Refs)

51. DNA REPAIR ASSAYS AS TESTS FOR ENVIRONMENTAL MUTAGENS. A REPORT OF THE U.S. EPA GENE-TOX PROGRAM.

Larsen KH, Brash D, Cleaver JE, Hart RW, Maher VM, Painter RB, Sega GA
Environmental Mutagen Information Center, Oak Ridge Natl. Lab., Oak Ridge, TN, 37830
Mutat Res; 98(3):287-318 1982

Repair replication (RR) assayed in isopycnic gradients, RR measured by benzoylated naphthoylated diethylaminoethyl (BND) cellulose, RR assayed by bromodeoxyuridine (BUdR) photolysis, alkaline elution assays, repair of single-strand breaks measured in alkaline sucrose gradients, and loss of adducts from DNA are reviewed. BND cellulose chromatography has not been widely adopted for measurement of RR, despite its relative ease and the fact that multiple samples can be processed simultaneously. The BUdR photolysis method is better suited for screening than most of the other DNA repair assays; it can be well controlled using internal standards, and, if standard repair intervals are used, produces data suitable for precise quantitative comparisons. Alkaline elution appears to be useful for identification of DNA-damaging agents if appropriate quantification is performed; it is sensitive, easy to perform and analyze, applicable both in vivo and in vitro studies, and could be used for simultaneous processing of multiple samples. The alkaline sucrose gradient technique for determining DNA repair appears to have limited usefulness for hazard evaluation. The chemicals tested in the various DNA repair assays are listed in the summary table with chemical classifications, and, where available, carcinogenicity assessments. Most of the chemicals used were known inducers of DNA damage and repair, and known mutagens and carcinogens. Of the 49 compounds listed, 23 are alkylating agents, and an additional 11 are polycyclic aromatic hydrocarbons. On theoretical grounds, evidence of DNA repair is a poor indicator of genetic risk. Thus, risk assessment based on repair data must be highly speculative. (130 Refs)

52. MEASUREMENTS BY FILTER ELUTION OF DNA SINGLE- AND DOUBLE-STRAND BREAKS IN RAT HEPATOCYTES: EFFECTS OF NITROSAMINES AND GAMMA-IRRADIATION.

Bradley MO, Dysart G, Fitzsimmons K, Harbach P, Lewin J, Wolf G
Merck Inst. Therapeutic Res., West Point, PA, 19486
Cancer Res; 42(7):2592-2597 1982

This work presents a filter elution method for measuring DNA single- and double-strand breaks in primary rat hepatocytes without radioactive labeling of DNA. We have studied the effects of a series of nitrosamines and of gamma-irradiation on DNA single- and double-strand break induction. The repair of DNA single-strand breaks in the hepatocytes was measured after treatment with 60Co, 1-methyl-1-nitrosourea, and N-nitrosodimethylamine. The hepatocytes were isolated by ethylene glycol-bis(beta-aminoethyl ether)-N,N'-tetra acetic acid-collagenase perfusion and had a mean viability of 91 +-4% (SD). The isolated cells were treated for varying lengths of time with nitrosamines in suspension culture in L-15 medium containing 10% fetal bovine serum. After treatment, the cells were chilled, loaded onto 2-um polycarbonate filters, and lysed in a 2% sodium dodecyl sulfate-proteinase K solution, pH 9.6. The DNA was eluted from the filter at either native or denaturing pH with fractions collected every 3 hr. The quantity of DNA in each fraction was determined by measuring the fluorescent product formed between it and diaminobenzoic acid after ethanol-sodium acetate precipitation and trapping of the DNA on 0.2-um polycarbonate filters. The results show that the carcinogens, N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosodipropylamine, N-nitrosodibutylamine, and 1-nitrosopiperidine all made dose- and time-related increases in the number of single-strand breaks in rat hepatocytes. N-Nitrosodiphenylamine produced small numbers of single-strand breaks. No double-strand breaks were formed by any of the nitrosamines. Single-strand breaks induced by N-nitrosodimethylamine were repaired very slowly relative to repair of either gamma-ray or 1-methyl-1-nitrosourea-induced

single-strand breaks. This system has many advantages for studying carcinogen metabolism and DNA damage in hepatocytes, one of the major target cells for many carcinogens. (Author abstract) (19 Refs)

53. CHEMICAL QUANTIFICATION OF UNSCHEDULED DNA SYNTHESIS IN CULTURED HEPATOCYTES AS AN ASSAY FOR THE RAPID SCREENING OF POTENTIAL CHEMICAL CARCINOGENS.

Althaus FR, Lawrence SD, Sattler GL, Longfellow DG, Pitot HC
(c/o Pitot) McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706
Cancer Res; 42(8):3010-3015 1982

Technical modifications of the quantitative determination of unscheduled DNA synthesis in cultured hepatocytes are described which allow for the rapid identification of potentially carcinogenic chemicals on a large-scale screening basis. The test is based on the biochemical quantification of [methyl3H] thymidine incorporation into DNA in the presence of hydroxyurea following isolation of nuclei from hepatocytes treated with the agent under study. This procedure ('nuclei procedure') eliminates most of the background radioactivity which otherwise obscures the stimulation of DNA repair synthesis by agents that induce a relatively weak response. By combining the nuclei procedure with a double-labeling technique, test results can be obtained within a few hr after exposure of hepatocytes to the test agents. A test series involving 41 agents confirmed the reliability of the nuclei procedure for the assay of DNA repair synthesis. In addition, chemicals which had yielded conflicting results previously in the autoradiographic hepatocyte DNA repair test, such as 4-acetylaminofluorene, or which had passed unrecognized in the previous in vitro tests, such as the potent liver carcinogen methapyrilene hydrochloride, scored clearly positive in our test protocol. (Author abstract) (27 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

54. RELATIONSHIP OF DNA TERTIARY AND QUATERNARY STRUCTURE TO CARCINOGENIC PROCESSES.

Lipetz PD, Galsky AG, Stephens RE
Dept. Radiology, Ohio State Univ., Columbus, OH, 43210
Adv Cancer Res; 36:165-210 1982

DNA superstructure as studied in nucleoids (permeabilized and protein-depleted nuclei) and possible correlations between modification of the tertiary (supercoiling) and quaternary (supercoiled domains) levels of organization and carcinogenic processes are reviewed. Evidence that the nucleoid technique can be used to quantify extremely low levels of DNA strand breaks (less than 1/4.4 x 10(10) daltons of DNA) is discussed. Chromatin origins of DNA supercoiling; physiological relevance of decreased nucleoid DNA supercoiling; novobiocin and nalidixic acid as probes of DNA supercoiling; prokaryotic DNA supercoiling; and eukaryotic DNA superstructure are discussed. Crown gall tumorigenesis, chronic lymphocytic leukemia, 12-O-tetradecanoyl-phorbol-13-acetate-induced alterations of DNA superstructure in normal human cells and chromatin damage and repair are addressed. The relationship of DNA superstructure to the processes of carcinogenesis, carcinogenic promotion, and Z DNA is discussed. DNA superstructure studies may lead to a resolution of the conflict between the idea that neoplastic transformation results from altered informational flow from DNA (as in somatic mutation) and the concept that membrane-associated events lead to malignant transformation. DNA superstructure could be responsive to both levels of control. (211 Refs)

55. ASPECTS OF THE MODE OF ACTION OF MITOMYCIN C.

Keller PJ
Purdue University, Lafayette, IN
Diss Abstr Int [B]; 42(11):4435-B 1982

The Streptomycete metabolite mitomycin C is a clinically useful antitumor antibiotic which has been shown to cross-link DNA after chemical reduction, and after exposure to acidic conditions. The present work, had three objectives. The first objective was the structure elucidation of novel reaction products arising upon reduction of mitomycin C in the presence of potassium ethyl xanthate in neutral aqueous solution. This work led to the characterization of four 1,2-cis-disubstituted-10-ethylxanthyl-7 aminodecarbamoyl-mitosenes which possess the following substituents at C1 and C2: (a) 1,2-cis-fused-1'-ethoxy-1'-mercaptothiazolidine, (b) 1-mercapto-2-thio-O-ethylcarbamate, (c) a fused epithiotrithiazocine, and tentatively (d) a 1-ethylxanthyl and a 2-amino group respectively. On the basis of high field 1H-NMR (nuclear magnetic resonance) analysis, a theoretical framework for the determination of the conformation and for the assignment, in favorable cases, of the relative stereochemistry in the saturated five-membered ring of mitosenes was developed in conjunction with the structure elucidation work. The second objective was the confirmation or reassignment of the 13C-NMR spectrum of mitomycin C. The third objective was the investigation of the interaction of mitomycin C with DNA. It was found that mitomycin C - DNA cross-links are stable to attack by potassium ethyl xanthate under reducing conditions. (no Refs)

56. BLEOMYCIN AND TALISOMYCIN SEQUENCE-SPECIFIC STRAND SCISSION OF DNA: A MECHANISM OF DOUBLE-STRAND CLEAVAGE.

Mirabelli CK, Ting A, Huang CH, Mong S, Crooke ST
Smith Kline and French Lab., P.O. Box 7929, Philadelphia, PA, 19101
Cancer Res; 42(7):2779-2785 1982

Computer analyses of DNA sequencing data obtained using various restriction fragments of pBR 322 DNA indicate that a trinucleotide sequence (-Pyr-G-C-) is the most preferred site for cleavage by the antitumor antibiotic bleomycin A2. Talisomycin A, a structurally related bleomycin analog, cleaved at the sequences-G-T/A- most preferentially. However, the presence of a pyrimidine at the 5' side of guanine at the cleavage site did not increase the probability of that site being cleaved by talisomycin. Using denaturing and nondenaturing polyacrylamide gel analyses of the drug-DNA reaction products, the sites of both single- and double-strand breaks have been localized and differentiated. The results indicate that a major determinant for location of a site-specific double-strand break is the production of two closely spaced sequence-specific single-strand breaks by the drugs on opposite strands of the DNA. A four-base pair sequence is proposed for the optimal sequence for bleomycin-induced double-strand breaks. (Author abstract) (26 Refs)

57. CLINICAL PHARMACOLOGY OF BLEOMYCIN CISPLATIN.

Evans WE, Yee GC, Crom WR, Pratt CB, Green AA
Clinical Pharmacokinetics Lab., St. Jude Children's Res. Hosp., 332 North Lauderdale, Memphis, TN, 38101
Drug Intell Clin Pharm; 16(6):448-458 1982

The mechanisms of action, pharmacokinetics, and clinical toxicities of bleomycin (BL) and cisplatin (CIS) are reviewed. Commercially available BL, Blenoxane, consists of 13 different BL species and is approved for the chemotherapy of squamous cell carcinomas, lymphoma, and testicular carcinoma, although antitumor activity has been noted in other malignancies. The drug's primary cytotoxic action is probably related to its excising effect on free bases after binding to DNA, resulting in single strand breaks. After iv bolus administration in adults, BL disappears in the serum in a biphasic pattern, with mean initial and terminal phase half-lives of 24 min and 4 hr. BL also undergoes significant metabolic conversion. Mucocutaneous lesions are the most common manifestations of BL toxicity. CIS is available as a water soluble complex containing a central platinum atom surrounded by two chloride and two ammonia moieties in the cos position. CIS has shown significant activity against a variety of adult and pediatric tumors. Major advances in curative management of testicular cancer have occurred using CIS in combination chemotherapy. CIS inhibits DNA synthesis, but its precise mechanism of action is unknown. It is not absorbed orally and is administered primarily by the iv route. Mean initial and terminal phase half-lives of approx 23 min and 44-67 hr have been calculated. CIS is nonenzymatically converted to several products which do not have antitumor activity. Major toxic effects caused by CIS include nephrotoxicity, nausea and vomiting, myelosuppression, and ototoxicity. (118 Refs)

58. ETHIDIUM BROMIDE CHANGES THE NUCLEASE-SENSITIVE DNA BINDING SITES OF THE ANTITUMOR DRUG CIS-DIAMMINEDICHLOROPLATINUM(II).

Tullius TD, Lippard SJ
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Proc Natl Acad Sci USA; 79(11):3489-3492 1982

Exonuclease III has been shown previously to reveal the binding sites of the antitumor drug cis-diamminedichloroplatinum(II) on DNA. Pretreatment of the same DNA with the intercalator ethidium bromide causes new platinum binding sites to be detected by the exonuclease III method. In particular, a 5' d(G6-C-G2)3' sequence in a 165-base-pair restriction fragment of plasmid pBR322 becomes a preferred site for exonuclease III-detectable cis-diamminedichloroplatinum(II) binding. This switching of nuclease-sensitive platinum binding to new sites by the influence of another drug, ethidium bromide, offers an explanation at the molecular level for the phenomenon of synergism in combination chemotherapy. (Author abstract) (19 Refs)

59. MECHANISM OF INTERACTION OF CC-1065 (NSC 298223) WITH DNA.

Swenson DH, Li LH, Hurley LH, Rokem JS, Petzold GL, Dayton BD, Wallace TL, Lin AH, Krueger WC
Upjohn Co., Kalamazoo, MI, 49001
Cancer Res; 42(7):2821-2828 1982

CC-1065 (NSC 298223), a potent new antitumor antibiotic produced by Streptomyces zelensis, interacts strongly with double-stranded DNA and appears to exert its cytotoxic effects through disruption of DNA synthesis. We undertook this study to elucidate the sites and mechanisms of CC-1065 interaction with DNA. The binding of CC-1065 to synthetic and native DNA was examined by differential circular dichroism or by Sephadex chromatography with photometric detection. The binding of CC-1065 with calf thymus DNA was rapid, being complete within 2 hr, and saturated at 1 drug per 7 to 11 base pairs. The interaction of CC-1065 with synthetic DNA polymers indicated a specificity for adenine- and thymine-rich sites. Agarose gel electrophoresis of CC-1065-treated supercoiled DNA showed that CC-1065 did not intercalate. Site exclusion studies using substitutions in the DNA grooves showed CC-1065 to bind primarily in the minor groove. CC-1065 did not cause DNA breaks; it inhibited susceptibility of DNA to nuclease S1 digestion. It raised the thermal melting temperature of DNA, and it inhibited the ethidium-induced unwinding of DNA. Thus, in contrast to many antitumor agents, CC-1065 stabilized the DNA helix. DNA helix overstabilization may be relevant to the mechanism of action of CC-1065. (Author abstract) (27 Refs)

60. FORMATION OF THE CROSS-LINK 1-[N(3)-DEOXYCYTIDYL],2-[N(1)-DEOXYGUANOSINYL]ETHANE IN DNA TREATED WITH N,N'-BIS(2-CHLOROETHYL)-N-NITROSOUREA.

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Cancer Res; 42(8):3102-3105 1982

The cross-linked dinucleoside, 1-[N3-deoxycytidyl],2-[N1-deoxyguanosinyl]ethane, has been isolated from DNA which has been exposed to N,N'-bis(2-chloroethyl)-N-nitrosourea. It is probable that this structure is responsible for the interstrand cross-linking observed previously by physical methods. The modification is unique in that it cross-links DNA through two base-pairing positions and probably arises through the transfer of a chloroethyl group to one of the bases followed by a second reaction of this group with the other strand of DNA. Initial attack could be at the N3 position of deoxycytidine, the N1 position of deoxyguanosine, or possibly the O6 position of deoxyguanosine. Attack at the O6 position of deoxyguanosine would require an internal cyclization with the N1 position of deoxyguanosine before secondary reaction with the N3 position of deoxycytidine but would explain resistance to N,N'-bis(2-chloroethyl)-N-nitrosourea in cells capable of removing substituents on the O6 position of guanine. (Author abstract) (12 Refs)

61. CELL SPECIFICITY IN HEPATOCARCINOGENESIS: PREFERENTIAL ACCUMULATION OF O(6)-METHYLGUANINE IN TARGET CELL DNA DURING CONTINUOUS EXPOSURE OF RATS TO 1,2-DIMETHYLHYDRAZINE.

Bedell MA, Lewis JG, Billings K C, Swenberg JA
Dept. Pathology, Chemical Industry Inst. Toxicology, Research Triangle Park, NC, 27709
Cancer Res; 42(8):3079-3083 1982

Concentrations of the major methylated DNA purines were determined in two liver cell populations of Fischer 344 rats during administration of 30 ppm 1,2-dimethylhydrazine (SDMH) in the drinking water for periods of up to 4 wk. quantitation of 7-methylguanine and O6-methylguanine (O6MG) was achieved by highly sensitive optical methods following separation of DNA bases by high-performance liquid chromatography. Overall alkylation as indicated by the con-

centration of 7-methylguanine was near max in both hepatocytes and liver nonparenchymal cells by the third day of SDMH administration. Similar amounts of 7-methylguanine were present in the two liver cell populations at seven of nine time points during 4 wk of exposure. In contrast, dramatic differences in the cumulative concentrations of O6MG were seen in the two cell populations. Nonparenchymal cells accumulated O6MG during the first 8 days of exposure and had up to a 30-fold greater concentration of this product than did the corresponding hepatocytes. In the hepatocytes, a rapid decline in O6MG concentration was observed between 1 and 3 days of exposure to SDMH. Thereafter, only low levels of this promutagenic lesion were present in hepatocytes. Exposure of rats to the same regimen of SDMH for up to 10 mo caused angiosarcomas in the livers of over 90% of the animals, while hepatocellular carcinomas occurred in only 40%. Thus, a strong correlation exists between the inability to repair O6MG and cell specificity for carcinogenesis. (Author abstract) (50 Refs)

62. MUTAGENICITY TESTS AND IN VITRO TRANSFORMATION ASSAYS ON TRIETHANOLAMINE.

Inoue K, Sunakawa T, Okamoto K, Tanaka Y

Biological Res. Section, Tochigi Res. Laboratories, Kao Soap Co., Ltd., 2606, Akabane, Ichikaimachi, Hagagun, Tochigi 321-34, Japan

Mutat Res; 101(4):305-313 1982

The mutagenicity and cell-transforming activities of triethanolamine were examined by measuring induction of DNA damage in Bacillus subtilis, mutagenicity in Salmonella typhimurium and Escherichia coli, production of chromosomal aberrations in Chinese hamster cells, and transformation of hamster embryo cells. Diethanolamine and monoethanolamine were also examined for induction of transformation. The results suggest that triethanolamine may not produce genotoxic effects by reaction with DNA and consequent induction of changes in DNA structure and function. The increased incidence of tumors induced in female mice by triethanolamine may be a secondary or indirect result of some specific cytotoxic effect. (20 Refs)

63. DNA-METHYLATION BY NITROSOCIMETIDINE AND N-METHYL-N'-NITRO-N-NITROSOGUANIDINE IN THE INTACT RAT.

Gombar CT, Magee PN

Fels Res. Inst., Temple Univ. Sch. Medicine, Philadelphia, PA, 19140

Chem Biol Interact; 40(2):149-157 1982

The ability of the nitroso derivative of the drug cimetidine to interact with cellular macromolecules in the intact rat was investigated. Radio-labeled nitrosocimetidine was shown to methylate DNA in a variety of tissues in the rat after oral administration. Radioactivity was also detected in the RNA and protein extracted from these same tissues. Methylation of DNA by the parent compound, cimetidine, as not detected in any of the tissues studied. For comparison, the DNA methylation produced by the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) dosed orally was measured. DNA alkylation by MNNG was found to be approx 2-36x greater than that produced by NC, varying with the tissues studied. The highest yield of DNA alkylation was found in the stomach for MNNG and the small intestine for nitrosocimetidine suggesting pharmacokinetic differences. (Author abstract) (23 Refs)

64. ROLE OF METHYLATION IN THE INDUCED AND SPONTANEOUS EXPRESSION OF THE AVIAN ENDOGENOUS VIRUS EV-1: DNA STRUCTURE AND GENE PRODUCTS.

Conklin KF, Coffin JM, Robinson HL, Groudine M, Eisenman R

Dept. Molecular Biology and Microbiology, Tufts Univ. Sch. Medicine, Boston, MA, 02111

Mol Cell Biol; 2(6):638-652 1982

A variant chicken embryo (number 1836) cell culture was examined by immunoprecipitation and gel electrophoresis. The culture contained endogenous avian provirus ev-1 and ev-6, and spontaneously expressed the ev-1 provirus. The cells released noninfectious virions containing Rous-associated virus type O-related 70S RNA and gag gene products but devoid of reverse transcriptase activity or antigens. EV-1 encoded particles also lacked envelope glycoproteins. DNA from the spontaneously expressing 1836 cell ev-1 provirus contained deoxyribonuclease I-hypersensitive sites in both long-term repeat sequences and the 5' flanking and gag gene sequences, the DNA also contained a pattern of methylation different from that of inactive ev-1 DNA. The finding of specific sites in 1836 ev-1 DNA that are differentially methylated only in expressed ev-1 DNA suggests that a certain pattern of methylation, possibly undermethylation, effects transcriptional activation. (50 Refs)

65. MOLECULAR AND CELLULAR MECHANISMS IN CARCINOGENESIS (MEETING ABSTRACT)

Rajewsky MF

Institut fur Zellbiologie (Tumorforschung), Universitat Essen (GH), Hufelandstrasse 55, D- 4300 Essen 1, W. Germany

Molecular and Cellular Mechanisms of Irradiation, March 17-19, 1982, Neuherburg, W. Germany. Gesellschaft fur Strahlen und Umweltforschung MBH 65 pp., 1982.

Structural modifications of DNA in cellular chromatin are primary events in the multistage process of tumorigenesis initiated by chemical carcinogens, ionizing radiation, and UV light. Among the various classes of chemical carcinogens, the alkylating agents have been particularly well characterized with regard to their reaction products in DNA, and sophisticated methodology (including the use of monoclonal antibodies) has been developed for the detection of specific alkylation products. It is not yet clear which molecular processes are preferentially affected, or set into motion, by carcinogen-modified DNA structures, and which alterations of cellular genetic programmes result in the expression of malignant phenotypes. Among the mechanisms to be considered are different types of mutations, altered patterns of DNA methylation or m-RNA processing, gene rearrangements and amplification, and possibly error-prone DNA repair. There is evidence indicating that in order to become malignantly transformed, cells must be proliferating (or have retained the capacity to re-enter the cell cycle; 'Go-cells') at the time of carcinogen exposure. Furthermore, a number of model systems of carcinogenesis indicate a cell type and differentiation stage-dependency of carcinogenic effect. Certain carcinogen-DNA adducts can be specifically recognized, removed and repaired by cellular enzymes which are expressed to varying degrees in different cell types and tissues, individuals, and species. The differential capacity of cells to repair carcinogen-modified DNA (besides intercellular variations in the metabolic conversion of chemicals to their ultimate reactive derivatives), may constitute one of the determinants for the probability of malignant transformation. (no Refs)

66. REARRANGEMENT IN RDNA-BEARING CHROMOSOMES IN CELL LINES FROM NEOPLASTIC CELLS.

Henderson AS, Megraw-Ripley S
Dept. Human Genetics and Development, Columbia Univ. Health Sciences, New York, NY, 10032
Cancer Genet Cytogenet; 6(1):1-16 1982

Cytological hybridization in situ and karyotypic analysis have been used to investigate positional changes in rDNA, and morphological changes in rDNA-bearing chromosomes, in a series of cell lines established from patients with various proliferative disorders. Characteristic features of chromosomes in these cell lines include extreme differences in the number of rRNA genes per locus, translocations involving rDNA, and distinct rearrangements of a large portion of the rDNA complex to a q-terminal position on acrocentric chromosomes. (Author abstract) (50 Refs)

67. THE ONCOGENIC CIRCLE CLOSES.

Rigby PW
Imperial Coll. Science and Technology, London SW7 2BZ, England
Nature; 297(5866):451-453 1982

Characterization of cellular oncogenes is reviewed. Oncogenes exhibit target specificity within a lineage. Recombinant DNA technics have been applied to the cloned human bladder carcinoma oncogene. The relationship between cellular oncogenes and proto-oncogenes (genes detected by transfection) has been studied. Retrovirus onc genes have been cloned and used to analyze DNA of NIH cells transformed by a variety of cellular oncogenes. The cloned human bladder carcinoma oncogene has been shown to be homologous to ras, the transforming gene of Harvey murine sarcoma virus. Mechanisms of oncogene activation and action are discussed. (19 Refs)

68. HUMAN EJ BLADDER CARCINOMA ONCOGENE IS HOMOLOGUE OF HARVEY SARCOMA VIRUS RAS GENE.

Parada LF, Tabin CJ, Shih C, Weinberg RA
Center for Cancer Res., Dept. Biology, Massachusetts Inst. Technology, Cambridge, MA, 02139
Nature; 297(5866):474-478 1982

Examination of homologies between retroviral oncogenes and transforming sequences defined by transfection reveals that the human bladder carcinoma (EJ) oncogene is homologous to the Harvey sarcoma virus oncogene (ras). Structural analysis limits the region of homology to a 3.0-kilobase SacI fragment of the EJ oncogene. Both EJ and ras DNA probes detect similar transcripts in transfectants derived from bladder carcinoma cell lines. (Author abstract) (42 Refs)

69. ISOLATION OF A TRANSFORMING SEQUENCE FROM A HUMAN BLADDER CARCINOMA CELL LINE.

Shih C, Weinberg RA
Dept. Molecular Biology, Massachusetts General Hosp., Bostin, MA, 02139
Cell; 29(1):161-169 1982

We have isolated the component of human bladder carcinoma cell DNA that is able to transform mouse fibroblasts. The oncogenic sequence was isolated initially from a lambda phage genomic library made from DNA of a transfected mouse cell carrying the human oncogene. A subcloned insert of 6.6 kb that carried transforming activity was amplified in the plasmid vector pBR322. The subcloned oncogene has been used as a sequence probe in Southern blot analyses. The oncogene appears to derive from sequences present in normal cellular DNA. Structural analysis has failed so far to reveal differences between the oncogene and its normal cellular homolog. The oncogene is unrelated to transforming sequences detected in a variety of other types of human tumor cell lines derived from colonic and lung carcinoma and from neuroblastoma. In contrast, the EJ bladder oncogene appears closely related to one that is active in the human T24 bladder carcinoma cell line. The oncogene appears to have undergone little, if any, amplification in several bladder carcinoma cell lines. (Author abstract) (35 Refs)

70. ORGANIZATION, DISTRIBUTION, AND STABILITY OF ENDOGENOUS ECOTROPIC MURINE LEUKEMIA VIRUS DNA SEQUENCES IN CHROMOSOMES OF MUS MUSCULUS.

Jenkins NA, Copeland NG, Taylor BA, Lee BK
Jackson Lab., Bar Harbor, ME, 04609
J Virol; 43(1):26-36 1982

The endogenous ecotropic murine leukemia virus DNA content and integration sites were characterized for 54 inbred strains and substrains of mice by restriction enzyme digestion, Southern blotting, and hybridization with an ecotropic murine leukemia virus DNA-specific probe. More than 75% of these strains carried endogenous ecotropic proviruses which were located in at least 29 distinct integration sites in chromosomes of Mus musculus. Fourteen of these proviruses have been assigned specific locus designations. Most, but not all, of the endogenous ecotropic proviruses were structurally indistinguishable by this analysis from the prototype AKR ecotropic virus, and the distribution of these proviruses followed known relationships among the inbred strains and substrains of mice. These results suggest that, in general, viral DNA integration preceded the establishment of inbred mouse strains and that these integrations are relatively stable. (Author abstract) (42 Refs)

71. MOLECULAR CLONING AND CHARACTERIZATION OF MURINE LEUKEMIA VIRUS-RELATED DNA SEQUENCES FROM C3H/HEN MOUSE DNA.

Roblin R, Young JM, Mural RJ, Bell TE, Ihle JN
Cancer Biology and Biological Carcinogenesis Program, Frederick Cancer Res. Center, Frederick, MD, 21701
J Virol; 43(1):113-126 1982

Ten murine leukemia virus (MuLV)-related DNA sequences were isolated from C3H/HeN mouse genomic DNA by cloning of EcoRI fragments in a Charon 4A vector. Detailed restriction endonuclease maps of four of the clones were developed by using AKR MuLV [32P]cDNA as a probe. C3H clone 14-9 contains approx seven kilobase pairs of MuLV-related DNA, one copy of an MuLV long terminal repeat-like sequence, and a region of flanking mouse DNA. C3H clones 34.2 and 36.1 contain approximately two kilobase pairs of MuLV-related DNA, one copy of a MuLV LTR-like sequence, and differing lengths of flanking mouse DNA sequences. C3H clone 8.13 was found to contain an insert of 5.7 kilobase pairs of MULV-related DNA with two long terminal repeat-like regions and sequences which are partially homologous to AKv-1. Comparison of the restriction endonuclease cleavage maps of these C3H clones with maps recently developed for ecotropic and xenotropic MuLV DNAs indicates that C3H clone 14-9 corresponds to the 5'-terminal portion of a genomic DNA sequence related to xenotropic MuLVs, whereas C3H clones

34.2 and 36.1 correspond to the 3' terminal portions of genomic DNA sequences related to xenotropic MuLVs. Clone 8.13 represents a deleted, xenotropic MuLV-related provirus. C3H clones 14-9, 34.2, 36.1, and 8.13 provide defined DNA sequence probes with which to characterize the organization and expression of endogenous MuLV-related DNA sequences in the mouse genome. (Author abstract) (31 Refs)

72. MOST OF THE MURINE LEUKEMIA VIRUS SEQUENCES IN THE DNA OF NIH/SWISS MICE CONSIST OF TWO CLOSELY RELATED PROVIRUSES, EACH REPEATED SEVERAL TIMES.

Steffen DL, Mural R, Cowing D, Mielcarz J, Young J, Roblin R
Worcester Foundation for Experimental Biology, Shrewsbury, MA, 01545
J Virol; 43(1):127-135 1982

The structure of the endogenous murine leukemia virus (MuLV) sequences of NIH/Swiss mice was analyzed by restriction endonuclease digestion, gel electrophoresis, and hybridization to an MuLV nucleic acid probe. Digestion of mouse DNA with certain restriction endonucleases revealed two classes of fragments. A large number of fragments (about 30) were present at a relatively low concentration, indicating that each derived from a sequence present once in the mouse genome. A smaller number of fragments (1 to 5) were present at a much higher concentration and must have resulted from sequences present multiple times in the mouse genome. These results indicated that the endogenous MuLV sequences represent a family of dispersed repetitive sequences. Hybridization of these same digested mouse DNAs to nucleic acid probes representing different portions of the MuLV genome allowed construction of a map of the sites where restriction endonucleases cleave the endogenous MuLV sequences. Several independent recombinant DNA clones of endogenous MuLV sequences have been isolated from C3H mice. Analysis of these sequences shows that they have the structure of MuLV proviruses. The sites at which restriction endonucleases cleave within these proviruses appeared to be similar or identical to the sites at which these nucleases cleaved within the MuLV sequences of NIH/Swiss mice. This identity was confirmed by parallel electrophoresis. We conclude that the apparently complex pattern of endogenous MuLV sequences of NIH/Swiss mice consists largely of only two kinds of provirus, each repeated multiple times at dispersed sites in the mouse genome. (Author abstract) (15 Refs)

73. ENVELOPE GENE SEQUENCES WHICH ENCODE THE GP52 PROTEIN OF SPLEEN FOCUS-FORMING VIRUS ARE REQUIRED FOR THE INDUCTION OF ERYTHROID CELL PROLIFERATION.

Linemeyer DL, Menke JG, Ruscetti SK, Evans LH, Scolnick EM
Dept. Biochemical Genetics, Merck Res. Lab., Rahway, NJ, 07065
J Virol; 43(1):223-233 1982

A series of insertion-deletion mutants was constructed in a molecularly cloned DNA copy of the Friend strain of spleen focus-forming virus (SFFV). The mutants were produced by inserting a synthetic oligonucleotide linker containing the recognition sequence of SalI endonuclease into several different locations of the SFFV DNA. Three classes of mutants were isolated: insertion-deletion mutants in the 5' half of the SFFV genome, in the long terminal repeat of the SFFV genome, and in the env gene of the SFFV genome. The env gene mutant has a deletion of sequences shared in common between the env gene of SFFV and the env genes of mink cell focus-inducing murine leukemia viruses. From analyses of the biological activity of the various mutants and a biologically active subgenomic SFFV DNA fragment described herein, we can deduce that the coding sequence encompassing the env gene of SFFV is required for the biological activity. This region, required for the pathogenic phenotype, cannot be larger than 1.5 kilobase pairs, a size only slightly more than that sufficient to encode the nonglycosylated precursor of the gp52 env gene product. (Author abstract) (45 Refs)

74. MOLECULAR CLONING AND SEQUENCE DETERMINATION OF A MURINE SARCOMA VIRUS GENOME (MEETING ABSTRACT).

Van Beveren C, Van Straaten F, Papkoff J, Lai MH, Muller R, Hunter T, Verma IM
The Salk Institute, P. O. Box 85800, San Diego, CA, 92138
In Vitro; 18(3,part2):314 1982

Moloney murine sarcoma virus (Mo-MSV), isolated from a Moloney murine leukemia virus (Mo-MLV)-induced mouse rhabdomyosarcoma, is a helper-dependent, C-type retrovirus which elicits the rapid appearance of sarcomas at the site of injection in mice. The analysis of its genome indicated that the 5' half was essentially the same as that of helper Mo-MLV, whereas the 3' end contained a major substitution of cellular DNA. The amount of viral transforming product(s) obtained in infection was so low as to preclude detection by standard means, and no tumor-specific antiserum was produced in tumor-bearing animals. Upon molecular cloning of infectious, unintegrated Mo-MSV double-stranded DNA genomes, the determination of the nucleotide sequence of the 5833-nucleotide genome was greatly facilitated. It was then possible to predict the amino acid sequence of the product of the transforming v-mosMo gene. Peptides corresponding to the N- and C- termini of the putative protein were synthesized and used to raise antibodies in rabbits. Immunoprecipitation with sera against the 12-amino acid C-terminus permitted the identification of a 37kd product of the v-mosMo gene in acutely infected NIH-3T3 cells. The product exists in two forms which differ in degree of phosphorylation. No protein kinase activity has been demonstrated to be associated with either form. (no Refs)

75. IDENTIFICATION OF A UNIQUE MOUSE MAMMARY TUMOR VIRUS IN THE BALB/CNIV MOUSE STRAIN.

Puma JP, Fanning TG, Young LJ, Cardiff RD
Chiron Corporation, Berkeley, CA, 95616
J Virol; 43(1):158-165 1982

We examined the genetic structure, in terms of restriction endonuclease recognition sites, of the milk-transmitted, low-oncogenic mouse mammary tumor virus (MuMTV) of the BALB/cNIV mouse strain. An analysis with EcoRI documented the presence of acquired cNIV proviruses in the mammary tumor DNAs of BALB/cNIV animals. A comparison of tumor DNAs digested with PstI showed that both the cNIV MuMTV and C3Hf MuMTV proviruses lacked the 4.3-and 1.1-kilobase pair fragments characteristic of C3H MuMTV patterns. An examination of mammary tumor and normal, non-mammary tissue DNAs with BamHI supported the idea that the cNIV MuMTV is identical to the C3Hf MuMTV and demonstrated that these two low-oncogenic proviruses are identical to the high-oncogenic C3H MuMTV provirus with respect to a pair of BamHI sites which define a 1.3-kilobase pair fragment. For each of the three MuMTV strains, we also mapped DNAs generated in isolated virions by reverse transcription of their genomic RNAs. Our results showed that cNIV and C3Hf

MuMTV are distinct entities by virtue of an additional PstI site within the cNIV long terminal repeat sequence. Another unique feature of cNIV MuMTV revealed by the analysis of virion-generated DNAs was the existence of a family of genomes within the cNIV population. We concluded that cNIV is distinct from its presumptive C3Hf MuMTV predecessor. (Author abstract) (26 Refs)

76. SEQUENCE OF THE LONG TERMINAL REPEAT AND ADJACENT SEGMENTS OF THE ENDOGENOUS AVIAN VIRUS ROUS-ASSOCIATED VIRUS 0.

Hughes SH
Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724
J Virol; 43(1):191-200 1982

An infectious unintegrated circular rous-associated virus (RAV-0) DNA was molecularly cloned, and the sequence of the long terminal repeat (LTR) and adjacent segments was determined. The sequence of the LTR was found to be very similar to that of replication-defective endogenous virus EV-1. Like the EV-1 LTR, the RAV-0 LTR is smaller than the LTRs of the oncogenic members of the avian sarcoma virus-avian leukosis virus group. There is, however, significant homology. The most striking differences are in the U3 region of the LTR, and in this region there are a series of small segments present in the oncogenic viruses which are absent in RAV-0. These differences in the U3 region of the LTR could account for the differences in the oncogenic potential of RAV-0 and the avian leukosis virus. The regions adjacent to the RAV-0 LTR were compared with the available avian sarcoma virus sequences. A segment of approx 200 bases to the right of the LTR (toward gag) is almost identical in RAV-0 and the Prague C strain of Rous sarcoma virus. The segment of RAV-0 which lies between the end of the env gene and U3 is approx 190 bases in length. Essentially this entire segment is present between env and src in the Schmidt-Ruppin A strain of Rous sarcoma virus. Most of this segment is also present between env and src in Prague C; however, in Prague C there is an apparent deletion of 40 bases in the region adjacent to env. In Schmidt-Ruppin A, but not in Prague C, about half of this segment is also present between src and the LTR. This arrangement has implications for the mechanism by which src was acquired. The region which encoded the gp37 portion of env appears to be very similar in RAV-0 and the Rous sarcoma viruses. However, differences at the very end of env imply that the carboxy termini of RAV-0, Schmidt-Ruppin A, and Prague C gp37s are significantly different. The implications of these observations are considered. (30 Refs)

77. ORGANIZATION OF THE EPSTEIN-BARR VIRUS DNA MOLECULE. II. FINE MAPPING OF THE BOUNDARIES OF THE INTERNAL REPEAT CLUSTER OF B95-8 AND IDENTIFICATION OF ADDITIONAL SMALL TANDEM REPEATS ADJACENT TO THE HR-1 DELETION.

Hayward SD, Lazarowitz SG, Hayward GS
Dept. Pharmacology and Experimental Therapeutics, Johns Hopkins Univ. Sch. Medicine, Baltimore, MD, 21205
J Virol; 43(1):201-212 1982

We used cloned BamHI fragments from Epstein-Bar virus strain B95-8 [EBV(B95-8)] DNA to obtain detailed restriction maps of the region of the genome adjacent to the large internal repeat cluster. These maps together with the results of hybridization experiments using a 3.1-kilobase repeat probe defined more precisely the location of the junctions between the internal repeat cluster and the flanking unique-sequence DNA. On one side (UL), the repeat sequences extended 600 +-80 base pairs (bp) into BamHI-Y; on the other side (Us), they extended 1,300 +-200 bp into BamHI-C. Therefore, EBV(B95-8) DNA contained a nonintegral number of 3.1-kilobase repeat units, namely, 12.6 copies. The mapping studies also revealed a second series of internal tandem repetitions in EBV(B95-8) DNA located within the BamHI-H fragment. This cluster comprised 11 copies of a 135-bp repeat unit which contained a single site for the NotI restriction endonuclease. Hybridization to these cloned EBV(B95-8) fragments using total EBV(HR-1) DNA as probe indicated that the deletion in EBV(HR-1) removed all 3,000 bp of unique-sequence DNA which lay between the large 3.1-kilobase and the small 135-bp repeat clusters. Thus, the deletion which destroyed the transforming ability in the EBV(HR-1) virus was bounded on either side by tandem repetitions. (Author abstract) (25 Refs)

78. DISCRETE REGIONS OF SIMIAN VIRUS 40 LARGE T ANTIGEN ARE REQUIRED FOR NONSPECIFIC AND VIRAL ORIGIN-SPECIFIC DNA BINDING.

Prives C, Barnet B, Scheller A, Khoury G, Jay G
Dept. Biological Sciences, Columbia Univ., New York, NY, 10027
J Virol; 43(1):73-82 1982

The nondefective adenovirus Type 2 (Ad2)-simian virus 40 (SV40) hybrid viruses, Ad2+ND2 and Ad2+ND4, have been used to determine which regions of the SV40 genome coding for the large tumor (T) antigen are involved in specific and nonspecific DNA binding. Ad2+ND2 encodes 45,000 Mr (45K) and 56,000 Mr (56K) T antigen-related polypeptides. The 45K polypeptide did not bind to DNA, but the 56K polypeptide bound nonspecifically to calf thymus DNA. Ad2+ND4 encodes 60,000 Mr (60K), 66,000 Mr (66K), 70,000 Mr (70K), 74,000 Mr (74K), and 90,000 Mr (90K) T antigen-related polypeptides, all of which bound nonspecifically to calf thymus DNA. However, in more stringent assays, where tight binding to viral origin sequences was tested, only the 90K protein specified by Ad2+ND4 showed specific high affinity for sequences at the viral origin of replication. From these results and previously published experiments describing the SV40 DNA integrated into these hybrid viruses, it was concluded that SV40 early gene sequences located between 0.39 and 0.44 SV40 map units contribute to nonspecific DNA binding, whereas sequences located between 0.50 and 0.63 SV40 map units are necessary for specific binding to the viral origin of replication. (Author abstract) (41 Refs)

79. MAPPING OF A DNA-BINDING DOMAIN OF SIMIAN VIRUS 40 T-ANTIGEN USING NON-DEFECTIVE ADENOVIRUS 2-SIMIAN VIRUS 40 HYBRID VIRUSES.

Montenarh M, Deppert W, Henning R
Dept. Biochemistry, Univ. Ulm, P.O. Box 4066, 7900 Ulm, W. Germany
FEBS Lett; 142(1):129-132 1982

In the attempt to localize a nonspecific DNA-binding region in the polypeptide chain of tumor antigen (T-Ag), a comparison was made between the calf thymus DNA-binding properties of simian virus 40 (SV40) T-Ag from SV40-transformed human cells and T-Ag related polypeptides coded for by Ad+ND2 and Ad2+ND4. The study suggests a DNA-binding domain inside the polypeptide chain of T-Ag located between maximally 0.53 and 0.39 SV40 DNA map units. (21 Refs)

80. SEQUENCE ORGANIZATION OF A VIRAL DNA INSERTION PRESENT IN THE ADENOVIRUS-TYPE-5-TRANSFORMED HAMSTER LINE BKH268-C31.

Westin G, Visser L, Zabielski J, Van Mansfeld AD, Petterson U, Rozijn TH
Dept. Microbiology, Biomedical Centre, Box 581, S-751 23 Uppsala, Sweden
Gene; 17(3):263-270 1982

The smaller viral insert present in the 268-C31 cell line was cloned using the lambda phage Charon 4A as a vector and the nucleotide sequence of the host-viral junctions. The organization of the flanking eukaryotic sequences was studied. Twenty clones out of 100,000 scored positive in the hybridization tests using intact Ad5 DNA as a probe. Results indicated the presence of many more viral insertions in the BHK268-C31 DNA than the two that could be detected by the conventional reassociation kinetic studies and blotting experiments. (26 Refs)

81. HERPESVIRUS SAIMIRI STRAIN VARIABILITY.

Desrosiers RC, Falk LA
Harvard Medical Sch., New England Regional Primate Res. Center, Southborough, MA, 01772
J Virol; 43(1):352-356 1982

Herpesvirus saimiri was isolated from 22 squirrel monkeys by cocultivation of peripheral lymphocytes with permissive owl monkey kidney monolayer cells. Comparison of virion DNA fragments produced from restriction endonuclease digestion was used as a sensitive measure of strain variability. Although all isolates contained similarities and common features, 19 of 22 were readily distinguished. Three of the isolates, however, were indistinguishable and possibly were related epidemiologically. Distinct subtypes of H saimiri were not evident by these criteria; Peruvian, Colombian, Guyanan, and Bolivian squirrel monkeys yielded isolates with characteristic features peculiar to the geographic region. Three of three colony-born squirrel monkeys that were tested yielded a strain of virus distinct from that obtained from the mother. In separate experiments, two of three animals chosen at random yielded a strain of virus different from that originally obtained 16 and 22 months previously; only one of the three animals examined yielded the same strain of virus 22 months after the original isolation. The degree of restriction endonuclease fragment variability among H saimiri strains appeared to be greater than previously observed for other herpesviruses. (Author abstract) (23 Refs)

82. MALIGNANT TRANSFORMATION OF HUMAN EMBRYO RETINOBLASTS BY CLONED ADENOVIRUS 12 DNA.

Byrd P, Brown KW, Gallimore PH
Cancer Res. Campaign Laboratories, Dept. Cancer Studies, Medical Sch., Univ. Birmingham, Birmingham B15 2TJ, England
Nature; 298(5869):69-71 1982

Isolation of a recombinant plasmid (pAsc2)-trans human embryo retinoblast (HER) cell line that contains many copies of the adenovirus Type 12 (Ad 12) transforming region, expresses virus-specified proteins, and following inoculation into athymic mice, forms tumors which resemble retinoblastomas is described. Primary cultures of human retinoblasts were transfected with pAsc2, and one transformed focus, identified 45 days after transfection, was isolated and established as a continuous cell line. The cell line has been designated Ad 12 HER1 and was the first reported Ad 12-transformed human cell line. Cytogenetic analysis revealed a pseudotetraploid human karyotype. The Ad 12 HER1 cell line was grown beyond the thirty-sixth tissue culture passage level and at no time has its growth entered a crisis phase. When Ad 12 HER1 cell DNA was digested with the restriction endonucleases BglII or SstI and subjected to Southern blotting analysis using P-labeled Ad 12 EcoRI-C DNA fragments as a probe, Ad 12 sequences were found on many DNA fragments whose sizes ranged from greater than 12 kilobase pairs (kb) to 2.2 kb. As these enzymes cleave host DNA sequences only, each fragment represented a separate insert of pAsc2 sequences. Results suggested that Ad 12 HER1 cells contain intact, integrated copies of viral and vector sequences and it is likely that some of the inserts contain tandem repeats of the entire recombinant plasmid. Histological analysis of the Ad 12 HER1-induced tumors showed them to consist of clusters of small cells with many mitotic figures characteristic of poorly differentiated human retinoblastomas. (29 Refs)

OTHER RELATED STUDIES

83. DNA EXCRETION BY HUMAN LYMPHOCYTES.

Fedorov NA, Ianeva IS
Central Res. Inst. Hematology and Blood Transfusion, Moscow, USSR
Usp Sovrem Biol; 93(2):171-182 1982

Literature data on the synthesis and excretion of DNA by human lymphocytes are reviewed. Both phytohemagglutinin (PHA)-stimulated and nonstimulated lymphocytes were shown to excrete DNA into culture medium. DNA synthesis was shown to take place in the acellular system. Treatment of stimulated lymphocytes with various proteases resulted in specific release of DNA sequences. Analysis of the reassociation kinetics indicated the selective replication of these DNA sequences during the logarithmic phase of cell growth. Significant increase of the blood level of DNA was observed in the patients with various neoplasms. Spontaneous human tumors and the ascitic fluid of animals with experimental tumors was shown to contain a nuclear factor (5S DNA-containing substance with marked immunosuppressive effect on immune lymphocytes in vitro). It was suggested that the nuclear factor contains single- and double-stranded regions and very short insertions of oligoribonucleotides. Analysis of DNA excretion by lymphocytes from the patients with chronic lymphoid leukemia showed marked stimulation of excretion after incubation with PHA. (75 Refs) (In RUS)

84. INTERCALATING SUBSTANCES DO NOT INDUCE SISTER-CHROMATID EXCHANGES (SCES) IN VIVO.

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Mutat Res; 104(4/5):261-266 1982

Acridine derivative, proflavine (PF), and phenothiazine derivatives, methylene blue (MB) and chlorpromazine (CPZ) were investigated in vivo (Chinese hamsters, 6-12 mo old) in the sister-chromatid exchange (SCE) test for the demonstration of a possible DNA-damaging effect. To evaluate the cellular proliferation, the distribution of metaphases of the first, second, and third generations were determined in 200 mitoses each. Cells that had replicated their DNA exclusively before the implantation of 5-bromodeoxyuridine could not be distinguished from the first metaphases, and cells that had gone through more than three cell cycles were counted as third mitoses. PF as well as MB and CPZ did no lead to a significant induction

of SCE in the bone marrow of Chinese hamster. CPZ was tested up to a concentration corresponding to 4x the therapeutic dose in man. PF and MB were tested in vivo up to the same concentration level without showing any effect. An increase of the dose was limited by the solubility of the substances. The distribution of the first, second, and third mitoses showed that the proliferation of the cells analyzed in the bone marrow was not delayed in comparison with the control. To exclude artifacts from the experimental procedure, cyclophosphamide (CP) was tested as a positive control. CP led to a pronounced increase in the SCE frequencies without impending the proliferation of the cells. (13 Refs)

85. MUTAGENIC RESPONSES OF FIVE INDEPENDENT GENETIC LOCI IN CHO CELLS TO A VARIETY OF MUTAGENS. DEVELOPMENT AND CHARACTERISTICS OF A MUTAGEN SCREENING SYSTEM BASED ON SELECTION FOR MULTIPLE DRUG-RESISTANT MARKERS.

Gupta RS, Singh B
Dept. Biochemistry, McMaster Univ., Hamilton L8N 3Z5, Canada
Mutat Res; 94(2):449-466 1982

Response of several well characterized genetic markers such as those involving resistance to thioguanine, emetine, ouabain, 5-6-dichlororibofuranosylbenzimidazole, and methylglyoxal bisguanylhydrazone towards a number of direct- and indirect-acting mutagens was assessed in proline-requiring Chinese hamster ovary (CHO) cell line. Direct-acting alkylating and DNA-adduct forming agents such as beta-propiolactone, ethyleneimine, N-nitrosomethylurea, 4-nitroquinoline-N-oxide, methyl methanesulfonate, and ethyl methanesulfonate; DNA intercalating and cross-linking agents such as ICR-170, acridine orange, ethidium bromide, mitomycin C, and actinomycin D; and chemicals requiring microsomal activation in other systems such as benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene, benzidine, and beta-naphthylamine were examined. The response of various agents was determined at only two or three concentrations which should have resulted in between 10-80% cell killing. Mutation frequencies of different genetic markers which were observed in the control and mutagen-treated cultures, as well as the mutagenesis significance ratio of various markers, which provide a measure of their relative mutagenic response are shown. Of the genetic loci, no one single locus showed higher response to all of the chemicals, instead, depending upon the chemical, specific loci were found to be more responsive than others. Results indicated that CHO cells possess significant capacity for the metabolic activation of many procarcinogens, and also underscore the merits of measuring the mutagenic response at multiple genetic loci in mutagen screening studies. (40 Refs)

86. THE EFFECT OF MONOCLONAL ANTIBODY-DIRECTED IMMUNOTOXINS AND IMMUNOMODULATORS ON CULTURED OSTEOGENIC SARCOMA CELLS (MEETING ABSTRACT)

Embleton MJ, Flannery GR, Pelham J, Rowland GF, Pihl A, Baldwin RW
Cancer Research Campaign Laboratories, University of Nottingham, Nottingham, England
Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

A monoclonal antibody (MoAb) raised against an osteogenic sarcoma cell line, 791T, was conjugated to various cytotoxic drugs and toxins, and to Lymphoblastoid Interferon (IFN) mainly using N-succinimidyl-3-(2-pyridyldithio)-proprionate. Conjugates were tested for cytotoxic properties on 791T and several other cross-reactive osteogenic sarcoma cell lines using radioisotopic assays to measure protein or DNA synthesis. Virtually all immunotoxin conjugates retained antibody binding activity for 791T cells, in most, cytotoxic activity was lost or diminished compared with the unconjugated agent. The least inactivated were Vindisine/MoAb and Ricin A chain/MoAb, for which the LD50 values were approximately 100-fold and 30-fold less than the parent compounds respectively. IFN/Moab conjugates did not directly affect the cells, but IFN alone also had only a marginal cytostatic effect. However, IFN/MoAb was highly effective in stimulating natural killer (NK) cell activity against 791T and other target cells. 791T cells, but not antigenically unrelated cells, preincubated with the conjugate induced activation of NK cells against 51Cr-labelled third party cells in mixed culture. These studies indicate that further development is required for the preparation of immunotoxins, but suggest an alternative approach towards antitumor therapy using indirectly acting MoAb-directed immunomodulators. (no Refs)

87. THE ROLE OF A SERUM DNA-BINDING PROTEIN (64DP) IN THE DIAGNOSIS OF MALIGNANT NEOPLASMS.

Mitomi T, Nakasaki H, Tajima T, Ogoshi K, Tsuda M, Katsunuma T
Dept. Surgery, Sch. Medicine, Tokai Univ., 259-11 Isehara, Japan
Int Adv Surg Oncol; 5:245-259 1982

Role of the DNA-binding protein with mol wt 64,000 dalton (64DP) as a tumor marker in diagnosing and monitoring patients with malignant disease is described. 64DP was measured with column sodium dodecyl sulfate-polyacrylamide gel electrophoresis and a single radial immunodiffusion method. The elevation of serum 64DP in 96 patients with malignant disease over that observed in normal controls or in patients with non-neoplastic disease appeared quite significant. Serum 64DP levels in 26 patients with stomach carcinoma were categorized according to clinical stages. No clear correlation was observed between the levels of 64DP and the clinical stage. Twenty-nine patients were subjected to serial determination of serum 64DP to monitor the response to treatment. Serum 64DP showed a drop approx 3 wk after resectional treatment. The 64DP level did not appear to decrease to normal value even after complete removal of the malignant lesion by radical gastrectomy. Eleven patients with recurrent stomach carcinoma were tested for 64DP. The level ranged from 31-220 ug/ml in these patients. Approx half of the patients did not show elevation of 64DP levels. Patients with carcinoma of other organs showed preoperatively high values of 64DP with subsequent decreases 3-4 wk after successful surgical treatment. Analysis of the amino acid composition of 64DP indicated that it is rich in leucine, glutamic acid, and aspartic acid. Amino acids in lower amounts were tryptophan and cysteine plus cystine. (14 Refs)

88. GROWTH FACTORS FOR CULTURED MAMMARY AND FIBROBLASTIC CELLS (MEETING ABSTRACT)

Rudland PS, Smith JA
Ludwig Institute, Royal Marsden Hospital, Sutton, Surrey, England
Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

The growth of the mammary gland is controlled by a series of interacting mammotrophic hormones. However these hormones are relatively inactive compared with serum or novel,

semi-purified growth factors (GF) in stimulating cell proliferation of cultured rat mammary gland epithelial cells. These GF's are of two types, a pituitary GF exerting systemic control and GF's from surrounding cells which may exert local control of mammary epithelial cell proliferation. The effects of the GF's can be amplified by certain mammotrophic hormones (eg insulin: I and hydrocortisone: HC). As in the mammary gland, GF's such as FGF, EGF, PGF2 alpha (fibroblast growth factor, epidermal growth factor, prostaglandin F2alpha) can stimulate DNA synthesis in quiescent 3T3 fibroblasts and this can be modified with I and HC. To explain the kinetics obtained with PGF2 alpha and FGF two operational signals are postulated, signal (1) initiates a lag phase and signal (2) determines the magnitude of the rate of cellular entry into the S phase of the cell cycle. Usually the rate of entry into S governs the rate of cell division, but when serum is completely removed additional components (eg transferrin) are required. During the lag phase the pathways generated by signal (1) and signal (2) have to interact causing increases in hypothetical protein(s) inside the cell. One of the altered properties of virally transformed cells is their lack of response to GF's. In the two cases examined by other groups the viral transforming proteins directly act on the putative intracellular pathways leading to increased cell proliferation rates. (no Refs)

89. TRAPPING OF DNA-REACTIVE METABOLITES OF THERAPEUTIC OR CARCINOGENIC AGENTS BY CARBON-14-LABELED SYNTHETIC POLYNUCLEOTIDES.

Mehta JR, Ludlum DB
(c/o Ludlum) Div. Oncology, Dept. Medicine, Albany Medical Coll., Albany, NY, 12208
Cancer Res; 42(8):2996-2999 1982

Many substances which do not react with DNA directly are metabolized into important DNA-modifying intermediates. We have devised a method for trapping these intermediates with 14C-labeled nucleosides contained in a synthetic polynucleotide. The polynucleotide structure protects the labeled nucleoside from metabolism; thus, it is unaltered when the polymer is incubated with a drug-metabolizing system. However, when the polymer is incubated with this system and a compound which can be metabolized into a reactive species, these intermediates are trapped by the 14C-labeled nucleoside and subsequently are detected as new peaks of radioactivity in a digest of the labeled polynucleotide. This system has been used to detect reactive intermediates of cyclophosphamide generated by a liver homogenate. (Author abstract) (11 Refs)

90. S AND G-2 TRANSIT IN RELATION TO DNA PLOIDY IN MIXED, POLYPLOID TUMOR POPULATIONS (MEETING ABSTRACT).

Sklarew RJ, Post J
University Medical Center, Goldwater Memorial Hospital, Roosevelt Island, NY, 10044
Program and Abstracts of the 6th Annual Meeting of the Cell Kinetics Society, March 19-21, 1982, Houston, Texas. The Cell Kinetics Society 38 pp., 1982.

Polyploid MCA rat sarcoma cultures were pulse-labelled with 3H-TdR (thymidine), grown in Colcemid for intervals of up to 18 hrs, and pulse-labeled with 14-CTdR prior to fixation. In autoradiographs the Feulgen DNA content and grain count were determined for G-2 and mitotic cells labeled with 3H-alone. A Quantimet 720 imaging system was used to automate the simultaneous measurements. The serial DNA distributions and labeling indices of 3H-labeled G-2 and mitotic cells suggested that 1) S-transit is prolonged with increasing DNA ploidy level over a four-fold ploidy range; 2) higher DNA ploidy is also associated with prolongation of G-2 transit; 3) subpopulations of higher DNA ploidy tend to leave the cycle in G-2 as cultures reach confluency; 4) whereas, co-existent diploid cells leave the cycle in G-1; 5) decreased S-transit (with increasing ploidy) is associated with lower average rates of DNA synthesis, as determined by mean 3H-TdR grain counts. (1 Ref)

91. PROGNOSTIC IMPLICATIONS OF PLOIDY AND PROLIFERATIVE ACTIVITY IN HUMAN SOLID TUMORS.

Barlogie B, Johnston DA, Smallwood L, Raber MN, Maddox AM, Latreille J, Swartzendruber DE, Drewinko B
Dept. Developmental Therapeutics, Univ. Texas System Cancer Center, M.D. Anderson Hosp. and Tumor Inst., Texas Medical Center, Houston, TX, 77030
Cancer Genet Cytogenet; 6(1):17-28 1982

Ploidy and cell cycle compartment distribution were measured by DNA flow cytometry in 261 patients with a variety of tumors. Eighty-one percent of all tumors were aneuploid, and 72% were hyperdiploid. Ploidy levels spanned a wide range from hypodiploid to hyperoctaploid with mean and median values coinciding at a near-triploid DNA content. The proportion of cells with G1 DNA content decreased with increasing hyperdiploid abnormality. While unrelated to biopsy site and to a number of host factors such as age, sex and race, both ploidy and cytokinetic parameters were markedly affected by histopathologic diagnosis. Patients with metastatic lung, breast, and GI cancer had higher ploidy levels than individuals with the corresponding primary tumors. Ploidy remained constant, and G1/0 proportions showed only minor variation by disease site and over a median observation time of 6 mo. Prognostic factor analysis was performed in the subgroup of patients studied within 6 mo from diagnosis. The adverse impact of low tumor G1/0 proportion on survival was lost as the proportional hazard analysis was extended to include diagnostic subgroups. Accounting for histopathologic diagnosis, stage of disease, ploidy, and the proportion of tumor G1/0 cells, the following sequence of adverse prognostic factors in order of their relative ranks was established: absence of breast cancer, hypertriploid DNA index and presence of metastatic disease. Our study demonstrates that DNA content-derived information on intrinsic tumor cell features pertaining to cytogenetics and cytokinetics may provide an objective means of biologically relevant cancer classification. (25 Refs)

92. RELATION BETWEEN DNA FEATURES AND PROLIFERATIVE ACTIVITY IN NON-HODGKIN LYMPHOMAS (NHL) (MEETING ABSTRACT).

Costa A, Mazzini G, Del Bino G, Silvestrini R
Istituto Nazionale Tumori, Via Venezian 1, 20133 Milano, Italy
Program and Abstracts of the 6th Annual Meeting of the Cell Kinetics Society, March 19-21, 1982, Houston, Texas. The Cell Kinetics Society 38 pp., 1982.

The flow cytofluorometric analysis of DNA content and autoradiographic determinations of 3H-thymidine labeling index (LI) was studied on 90 NHL from adult patients, 75 untreated patients at the time of diagnosis and 15 previously treated patients at the time of relapse. The frequency distributions of diploid and aneuploid tumors were similar in untreated and previously treated patients. Fifty-seven of 90 tumors had aneuploid DNA content; 30% of these were heterogeneous cell populations. A wide range of proliferative activity was ob-

served in diploid and aneuploid tumors (0.01-15.9%, 0.01-30%, respectively) with a statistically different median value (1.35% vs 3%, p less than 0.01). About 60% of nodular lymphomas were aneuploid, and no kinetic difference was observed between diploid and aneuploid groups. About 75% of diffuse lymphomas were aneuploid. A higher median LI was observed for this group than for the diploid one (6.25% vs 1.35%), but the difference was not statistically significant. The DNA characteristics and LI will be analyzed in relation to response to treatment and survival to assess their clinical relevance. (no Refs)

93. DNA CONTENT AS A MARKER OF PRE-CANCEROUS LESIONS OF THE DIGESTIVE TRACT (MEETING ABSTRACT).

Capurso L, Teodori L, DeVita R, Galloni L, Tarquini M, Mauro F, Nervi C, Pallone F
Cattedra Gastroenterologia Universita, Rome, Italy
Gastroenterology; 82(5,part2):1029 1982

In order to detect the abnormality of the DNA content distribution as a marker of precancerous or early cancerous lesions of the digestive tract, human endoscopic biopsies have been analyzed. Samples were derived from stomach, duodenum and colon of normal donors and of patients clinically classified as affected by non-neoplastic lesions such as ulcer, atrophic gastritis, chronic gastritis and polyps. Sample processing has been performed by mechanical and enzymatic treatment (collagenase and haluronidase vs pepsin treatment revealed no significant changes in the DNA pattern). Staining for flow cytometric analysis (Phywe Ortho ICP 22) has been achieved by ethidium bromide and mithramycin. Normal samples exhibited the usual DNA distribution with G1/0,S and G2/M compartments. An aneuploid cell subpopulation was found in 4/7 atrophic or chronic gastritis specimens. Such aneuploidy is similar to that typical of gastric adenocarcinoma, DNA index ranging from 1.1 to 1.4 in comparison to 1.0. However, the percent aneuploid cells in the majority of tumors was generally higher than in aneuploid gastrites. Furthermore, polyps also exhibited an aneuploid peak, while ulcers were characterized by a normal, diploid DNA content. It is likely that the occurrence of aneuploidy in non-neoplastic lesions reflects some pre-cancerous situation or the presence of an early, small fraction of neoplastic cells. (no Refs)

94. QUANTITATIVE EVALUATION OF STRUCTURAL CHANGES OF CELL NUCLEI IN VARIOUS PATHOLOGICAL STATES OF THE CERVIX UTERI.

Zheleznov BI, Ezhova LS
All-Union Res. Center Motherhood and Childhood Protection, Moscow, USSR
Akush Ginekol (Mosk); (4):51-55 1982

The results of cytospectrophotometric analysis of biopsy or surgical specimens from 25 women with various pathologic changes in the cervix uteri are reported. Av DNA contents of the specimens showed progressive increases from 51.9 units in normal basal epithelium to 78.65 in pseudoerosion with metaplasia, 106.17 in severe dysplasia, and 112.26 in preinvasive carcinoma; the value in mild dysplasia was 45.86 units. The mitotic index in preinvasive carcinoma cells was 19.5 milli%, compared with 4.0 in basal epithelium, 3.75 in metaplastic epithelium, 7.33 in mild dysplasia, and 13.8 in severe dysplasia. A characteristic feature of specimens of preinvasive carcinoma was a marked increase in the number of pathologic mitoses (48.3%). (12 Refs) (In RUS)

95. TUMOR CELL TRANSFERRIN RECEPTOR NUMBER: AN INDICATOR OF GROWTH STATE (MEETING ABSTRACT).

Rasey JS, Larson SM, Nelson NJ
University of Washington, Seattle, WA, 98195
Program and Abstracts of the 6th Annual Meeting of the Cell Kinetics Society, March 19-21, 1982, Houston, Texas. The Cell Kinetics Society 38 pp., 1982.

Uptake of iron and other trace metals by several tumor and normal cells occurs via cell surface transferrin receptors. Iron is essential for DNA synthesis in some cells types; this is consistent with larger numbers of transferrin receptors per cell in actively proliferating compared to confluent plateau phase tissue cultures. We have determined relative number of transferrin receptors/cell and receptor affinity for transferrin in EMT-6 and RIF-1 sarcoma cells in vitro. For both cell types, receptor number begins to increase prior to the initiation of cell division, increases markedly during early exponential growth, and decreases prior to attainment of a plateau in cell number. Affinity does not change. Radiation-induced progression delay in exponentially growing cells is being used to determine if the receptor number/cell remains high in growth inhibited cultures, or, conversely, if the growth inhibition is mediated through down-regulation of receptor number. If the latter is true, assay of receptor number by binding of I-125 transferrin may be a useful kinetic technique. (no Refs)

96. QUANTITATIVE CHANGES IN ESTROGEN RECEPTOR PRODUCED BY CHRONIC DES TREATMENT OF TWO MOUSE STRAINS DIFFERING IN SUSCEPTIBILITY TO LEYDIG CELL TUMOR INDUCTION.

Terakawa N, Huseby RA, Fang SM, Samuels LT
Dept. Obstetrics and Gynecology, Osaka Univ. Medical Sch., 1-1-50 Fukushima, Fukushima-ku, Osaka, Japan
J Steroid Biochem; 16(5):643-652 1982

The effects of chronic diethylstilbestrol (DES) treatment on the estrogen receptor system in testes of BALB/c mice, which develop Leydig cell tumors after long-term DES, and C3H mice, which do not, were investigated. Estrogen receptor concentration in Leydig cell cytosols from C3H mice was similar to that observed in BALB/c mice at the beginning of DES treatment, but changed during long-term estrogenization. Estrogen receptor sites in cytosol increased significantly in BALB/c mice during DES treatment without any alteration in affinity for estrogen. A similar but lesser increase was observed in C3H mice. A single DES treatment caused an increase of nuclear receptor content in BALB/c mice which was greater than that observed in C3H mice. In C3H testes the nuclear estrogen receptor content remained constant after 7-14 days treatment while that in BALB/c mice rose to a level 150% that observed 0.75 hr after a single injection of 2.5 ug DES. These differences in nuclear receptors parallel differences in both early induction of DNA synthesis in Leydig cells and the induction of Leydig cell tumors observed in the two strains. (26 Refs)

97. CYTOLOGICAL AND CYTOGENETICAL STUDIES ON HUMAN MENINGIOMA.

Zang KD
Inst. Human Genetics, Univ. Saar, 6650 Homburg, W. Germany
Cancer Genet Cytogenet; 6(3):249-274 1982

Characteristics of meningiomas which make them a model system for the study of human cancer development are described. Meningiomas show a remarkably good correlation between karyotype and histological and growth patterns which particle cultures of the individual tumors develop in vitro. Histological classification of meningiomas is described. In contrast to true brain tumors, meningiomas occur most often in the fifth and sixth decade of the life and occur twice as frequently in females as in males. Characterization of meningiomas according to their respective karyotype shows a highly significant shift in sex distribution. In tumors with normal karyotypes, the male:female sex ratio is 1:3 while in more aberrant forms the ratio is approx 1:1. Chromosomal indications for virus involvement in the etiology of meningiomas are presented along with immunofluorescence data, induction of virus particles, and demonstration of episomal Simian virus 40 (SV40) DNA in meningiomas. Experimental transformation of meningioma cell cultures and fibroblast cultures of the same patients with SV40 is described. (62 Refs)

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EFFECT OF EXOGENOUS AND ENDOGENOUS AGENTS ON DNA SYNTHESIS

1. INFLUENCE OF GLYCOSAMINOGLYCANS ON ENDOGENOUS DNA SYNTHESIS IN ISOLATED NORMAL AND CANCER CELL NUCLEI. DIFFERENTIAL EFFECT OF HEPARIN.

Furukawa K, Bhavanadan VP
Dept. Biologicl Chemistry, M. S. Hershey Medical Center, Pennsylvania State Univ., Hershey, PA, 17033
Biochim Biophys Acta; 697(3):344-352 1982

The influence of exogenously-added glycosaminoglycans and glycoproteins on DNA synthesis in isolated nuclei, from normal and malignant tissues, was investigated. Heparin stimulated DNA synthesis in normal cell nuclei at concentrations (heparin/DNA (w/w) less than 0.9) which inhibited DNA synthesis in tumor cell nuclei. At higher concentrations (heparin/DNA (w/w) greater than 0.9) heparin inhibited DNA synthesis in both normal and tumor cell nuclei. The chondroitin-4 and 6-sulfates, heparan sulfate, cartilage proteogl/can, N-desulfated heparin, and glycophorin caused inhibition of DNA synthesis at all concentrations tested and in all nuclei examined. Hyalur-

HE 20.3173/2:

onic acid, dermatan sulfate, heratan sulfate, alpha1-acid glycoprotein and fetuin had no significant influence on DNA synthesis in isolated nuclei. (Author abstract) (42 Refs)

2. NEWLY SYNTHESISED DNA IN AGEING HUMAN CELLS IN CULTURED TREATED WITH 4-NITROQUINOLINE-1-OXIDE.

Cooke A, Harris WJ
Dept. Medical Genetics, Royal Hosp. for Sick Children, Yorkhill, Glasgow, Scotland
Mech Ageing Dev; 19(2):171-180 1982

Two lines of normal human embryonic lung fibroblasts, MRC-5 and F2002, were serially subcultured until senescence was attained. When cells were exposed to varying concentrations of the chemical carcinogen 4-nitroquinoline-1-oxide (4-NQO), the rate of DNA synthesis (as measured by thymidine incorporation) was reduced in a dose-dependent fashion in cells from both early and late passages. While the overall amount of incorporation was considerably lower in old cells, the extent of inhibition caused by 4-NQO treatment (relative to appropriate controls) was not related to culture age. Alkaline sucrose density gradient analysis of newly synthesised DNA from cells pretreated with 4-NQO failed to detect any significant variation in the size of labeled DNA from cells examined immediately after incubation with radioactive thymidine. The shift of this labeled material to high mol wt in 4-NQO-treated cells also showed no age-related difference. (Author abstract) (26 Refs)

3. BIOCHEMICAL RESPONSE OF LYMPHOMA CELLS TO MITOGENIC STIMULATION BY PROLACTIN.

Richards JF, Beer CT, Bourgeault C, Chen K, Gout PW
Dept. Biochemistry, Univ. British Columbia, Vancouver V6T 1W5, Canada
Mol Cell Endocrinol; 26(1/2):41-49 1982

The biochemical events involved in the mitogenic stimulation of cells by prolactin (PRL) at physiological concentrations were investigated in cultures of Nb 2 node rat lymphoma cells. After the addition of PRL (20 nanog/ml) at 18 hr to a stationary culture, cell numbers remained stable for approx 22 hr, then doubled. DNA synthesis remained low during the first 10 hr after addition of PRL, but rapidly increased to a peak at approx 17 hr. A second wave of DNA synthesis was then seen. Activity of ornithine decarboxylase (ODC) was measured in control cultures and cultures treated with PRL. Enzymatic activities of stationary cells was very low, while that of replicating cells was more than 100-fold higher. In the presence of PRL, ODC activity was substantially higher than that of cells growing in medium containing fetal calf serum. Addition of other pituitary hormones to stationary cultures had no effect on ODC activity, demonstrating that the increase in ODC activity was due to PRL per se. Addition of PRL resulted in striking increases of ODC and S-adenosyl methionine decarboxylase activities: each enzyme exhibited two peaks of activity, the second of which appeared to coincide with the increase in the rate of DNA synthesis. In the absence of PRL, cells accumulated early in the G1 phase, while PRL induced the cells to proceed through the cell cycle in a synchronized fashion. Thus, lymphoma cells are useful for studying biochemical events resulting from interaction of a mitogenic polypeptide hormone and its target cell. (24 Refs)

4. FETAL AND ONCOFETAL ANTIGENS. REGULATORS OF IMMUNE REACTIONS.

McCluskey DR
Dept. Haematology, Royal Victoria Hosp., Belfast, Northern Ireland
Med Hypotheses; 8(6):627-634 1982

Cellular immune system, fetal and oncofetal antigens, and the maternal-fetal relationship is discussed. Neoplastic transformation is associatied with expression of neoantigens, which are capable of stimulating an immune response, but also with re-expression of fetal antigens which appear to activate a variety of specific and non-specific suppressor mechanisms. In pregnant females and patients with choriocarcinoma, peripheral blood lymphocytes inhibited DNA synthesis of normal lymphocytes thus suggesting that in pregnancy and patients with trophoblastic tumors, altered lymphocyte immunocompetence may play a role in survival of the fetus and neoplasm respectively. (44 Refs)

5. PERSPECTIVES ON INTERACTIONS OF ACYCLOVIR WITH EPSTEIN-BARR AND OTHER HERPES VIRUSES.

Pagano JS, Datta AK
Sch. Medicine, Univ. North Carolina, Chapel Hill, NC, 27514
Am J Med; 73(1A):18-26 1982

Acyclovir [9-(2-hydroxyethoxymethyl)guanine] inhibits Epstein-Barr virus (EBV) replication in lymphoblastoid cells at concentrations nontoxic to cellular growth. The mode of action of the drug against EBV differs from the mechanism described in herpes simplex virus systems. Due to the absence of virus-specified thymidine kinase, the drug is poorly phosphorylated in EBV-infected cells. The extent of monophosphorylation is similar both in mock-infected and EBV-infected cells. Despite weak phosphorylation of the drug, the replication of linear EBV DNA is inhibited due to exquisite sensitivity of the viral DNA polymerase. Activation of acyclovir does not require phosphorylation by virus-specified thymidine kinase. Inhibition of different herpes-group viruses depends on three variable factors: degree of phosphorylation, cellular metabolism of the drug, and degree of sensitivity of the viral polymerase. Interaction of acyclovir-triphosphate with EBV DNA polymerase is reversible. Cells infected with EBV and treated with acyclovir resume virus replication following removal of the drug even after long exposure. Acyclovir inhibits replication of linear genomes and stops production of virus, but has no effect on latent cellular infection. These results lead us to predict that acyclovir will suppress, but not cure, EBV infection. (Author abstract) (33 Refs)

6. ACYCLOVIR INHIBITION OF VIRAL DNA CHAIN ELONGATION IN HERPES SIMPLEX VIRUS-INFECTED CELLS.

McGuirt PV, Furman PA
Dept. Experimental Therapy, Wellcome Res. Lab., Research Triangle Park, NC, 27709
Am J Med; 73(1A):67-71 1982

The effect of acyclovir on DNA synthesized in HSV-1-infected cells was examined. Viral DNA synthesis was significantly reduced in the presence of 10 uM acyclovir monitored by cRNA-DNA hybridization and isopycnic centrifugation in cesium chloride. DNA synthesized in infected cells in the presence of acyclovir appeared to be chain-terminated when evaluated by rate zonal centrifugation in alkaline sucrose. Cellular DNA synthesis in actively replicating uninfected cells in the presence of acyclovir was evaluated by isopycnic centrifuga-

tion. Cellular DNA synthesis appeared unaffected at concentrations of acyclovir up to 100 uM. (Author abstract) (7 Refs)

7. EFFECT OF ACYCLOVIR ON HERPES SIMPLEX VIRUS REPLICATION IN A PERSISTENTLY INFECTED HUMAN LYMPHOBLASTOID CELL LINE (P3HR-1).

Shaw JE, Colby BM, Leong J, Bolling TJ

Univ. North Carolina, Box 3, Swing Building, 217H, Chapel Hill, NC, 27514

Am J Med; 73(1A):72-76 1982

We examined the effect of acyclovir on herpes simplex virus (HSV) replication in the persistently infected human lymphoblastoid cell line, P3HR-1. During 10 days of continuous exposure of P3HR-1 cells to 100 uM acyclovir there was a decrease in the number of cells expressing HSV antigens, the number of copies of HSV DNA per cell, and the infectivity of extracellular virus. A significant increase in each of these was detected after 14 days of continuous exposure of P3HR-1 cells to the drug, demonstrating that the replication of HSV has become resistant to inhibition by acyclovir. No apparent resistance of EBV replication to inhibition by acyclovir, however, was detected in HSV-infected P3HR-1 cells. The number of copies per cell of EBV and HSV DNA increased after exposure of HSV-infected P3HR-1 cells to the tumor promoter TPA (12-O-tetradecanoyl-phorbol-13-acetate). When cells were exposed continuously to acyclovir for 4 mo and then exposed to acyclovir and TPA simultaneously, the number of copies per cell of HSV DNA increased, but the number of copies per cell of EBV DNA increased by only a few, if any. The detection of P3HR-1 cultures of individual cells expressing both HSV and EBV antigens suggested that HSV and EBV productive cycles could occur simultaneously within the same cell. However, single cells very rarely expressed both antigens simultaneously. (Author abstract) (19 Refs)

8. EFFECT OF 9-(2-HYDROXYETHOXYMETHYL)GUANINE ON VIRAL-SPECIFIC POLYPEPTIDE SYNTHESIS IN HUMAN CYTOMEGALOVIRUS-INFECTED CELLS.

Mar EC, Patel PC, Huang ES

Cancer Res. Center, Sch. Medicine, Univ. North Carolina, Chapel Hill, NC, 27514

Am J Med; 73(1A):82-85 1982

Plaque-forming assay resulted in a 50% inhibitory dose by 9-(2-hydroxyethoxymethyl)guanine (acyclovir) against Towne strain human cytomegalovirus (HCMV) of approximately 98 umol. At the drug concentration of 200 umol, we did not detect any significant inhibition of viral DNA synthesis by cRNA-DNA hybridization. However, at this drug concentration, the synthesis of at least two viral-specific late polypeptides (150K and 67K) was significantly retarded up to 48 hr after infection, but resumed at 72 hr. These data suggest that acyclovir or its in vivo transformed derivative had a transient effect on viral-specific polypeptide synthesis in HCMV-infected human fibroblasts at a high drug concentration. (Author abstract) (19 Refs)

9. INHIBITION OF DNA REPLICATION AND GROWTH OF SEVERAL HUMAN AND MURINE NEOPLASTIC CELLS BY APHIDICOLIN WITHOUT DETECTABLE EFFECT UPON SYNTHESIS OF IMMUNOGLOBULINS AND HLA ANTIGENS.

Pedrali-Noy G, Belvedere M, Crepaldi T, Focher F, Spadari S

Istituto di Genetica Biochemica ed Evoluzionistica, Consiglio Nazionale delle Ricerche, Via S. Epifanio, 14 27100 Pavia, Italy

Cancer Res; 42(9):3810-3813 1982

Aphidicolin inhibits DNA replication and growth of all tested human and murine neoplastic cells including leukemic T- and B-lymphocytes and melanocarcinoma cells. The concentration of aphidicolin causing 50% inhibition of DNA synthesis in all of the tested neoplastic cell lines is similar to that necessary to inhibit DNA synthesis in HeLa cells by 50%. The mechanism of inhibition of DNA synthesis in neoplastic cells is again due to the inhibition of DNA polymerase alpha by aphidicolin. Aphidicolin at a concentration 100 times higher than that causing 50% inhibition of DNA synthesis and cell growth had no effect on total protein synthesis, on the secretion of immunoglobulins, or on the expression of HLA antigens which are involved in relevant phenomena of the immune response. (Author abstract) (28 Refs)

10. INHIBITION OF DNA REPLICATION AND REPAIR BY ANTHRALIN OR DANTHRON IN CULTURED HUMAN CELLS.

Clark JM, Hanawalt PC

Dept. Biological Sciences, Stanford Univ., Stanford, CA, 94305

J Invest Dermatol; 79(1):18-22 1982

The comparative effects of the tumor promoter anthralin and its analog, danthron, on semiconservative DNA replication and DNA repair synthesis were studied in cultured human cells. Bromodeoxyuridine was used as density label together with 3H-thymidine to distinguish replication from repair synthesis in isopycnic CsCl gradients. Anthralin at 1.1 uM inhibited replication in T98G cells by 50%. In cells treated with 0.4 or 1.3 uM anthralin an additive effect was observed on the inhibition of replication by ultraviolet light (254 nm). In cells irradiated with 20 J/m2, 2.3 uM anthralin was required to inhibit repair synthesis by 50%. Thus there was no selective inhibitory effect of anthralin on repair synthesis. Danthron exhibited no detectable effect on either semiconservative replication or repair synthesis at concentrations below about 5.0 uM. Neither compound stimulated repair synthesis in the absence of ultraviolet irradiation. Thus, anthralin and danthron do not appear to react with DNA to form adducts that are subject to excision repair. Although both compounds appear to intercalate into supercoiled DNA in vitro to a limited extent, the degree of unwinding introduced by the respective drugs does not correlate with their relative effects on DNA synthesis in vivo. Therefore the inhibitory effect of anthralin on DNA replication and repair synthesis in T98G cells does not appear to result from the direct interaction of the drug with DNA. (Author abstract) (20 Refs)

11. PLATINUM-COMPLEXED ANTITUMOR IMMUNOGLOBULINS THAT SPECIFICALLY INHIBIT DNA SYNTHESIS OF MOUSE TUMOR CELLS.

Hurwitz E, Kashi R, Wilchek M
Dept. Biophysics, Weizmann Inst. Science, Rehovot 76100, Israel
JNCI; 69(1):47-51 1982

Platinum salts were complexed with antitumor reactive immunoglobulins (Ig). The Pt-complexed antitumor Ig retained their specific antibody binding activity. The inhibition of DNA synthesis by Pt salts (K2 PtCl4 and cis-diamminedichloroplatinum II) and Pt-Ig complexes was studied in two tumor cell lines of mice. The Pt-Ig complexes were much more effective inhibitors of DNA synthesis when complexed with Ig, which had binding affinity toward the test tumor cells. (Author abstract) (27 Refs)

12. INHIBITION OF DNA SYNTHESIS IN MURINE TUMOR CELLS BY GELDANAMYCIN, AN ANTIBIOTIC OF THE BENZOQUINOID ANSAMYCIN GROUP.

Yamaki H, Suzuki H, Choi EC, Tanaka N
Inst. Applied Microbiology, Univ. Tokyo, Tokyo 113, Japan
J Antibiot (Tokyo); 35(7):886-892 1982

The mechanisms of action of geldanamycin, a benzoquinoid ansamycin, was investigated with murine lymphoblastoma L5178Y cells. The agent inhibited the cell growth at concentrations over 0.01 ug/ml. The antibiotic blocked DNA synthesis more markedly than RNA and protein syntheses. Mitosis was not significantly affected by the drug in the cells synchronized with demecolcine (Colcemid). The antibiotic did not interfere with in vitro assembly of tubulin. In the synchronized cells, strong inhibition of DNA synthesis was observed when geldanamycin was introduced into the culture prior to S phase of the cell cycle. The degree of inhibition was stronger with prolongation of incubation period and with increase of DNA synthesis rate. The results suggested that initiation of DNA synthesis or S phase is affected by the drug. DNA degradation was not significantly induced in vivo by the antibiotic. Geldanamycin blocked DNA polymerase alpha more markedly than beta and gamma. The degree of inhibition depended upon concentrations of enzyme but not upon those of template, suggesting a drug-enzyme interaction IC50 for DNA polymerase alpha was 10 ug/ml and for DNA polymerase beta 100 ug/ml at low concentrations of enzyme. The inhibition of DNA polymerase alpha by the antibiotic was non-competitive and Ki was 20 uM. (Author abstract) (16 Refs)

13. EFFECTS OF OLIVACINE ON THE METABOLISM OF PROTEINS AND NUCLEIC ACIDS IN ESCHERICHIA COLI.

Touaty P, Simon M
Laboratoire d'Enzymologie Microbienne, C.N.R.S., 91190 Gif-sur-Yvette, France
Biochim Biophys Acta; 697(3):313-321 1982

Olivacine, a semi-synthetic isomer of ellipticine, interferes with the growth of Escherichia coli. Investigation of the effects of graded concentration shows that, in vivo, the synthesis of overall proteins is markedly more sensitive to low concentrations than is the synthesis of RNA and DNA, respectively. Furthermore, at moderate concentrations, olivacine is much more inhibitory to the synthesis of cytoplasmic proteins than to that of envelope proteins. At lethal concentration (10(-4)) all macromolecular syntheses are inhibited, DNA synthesis, however, to a lower extent. Olivacine blocks the synthesis of polyribonucleotide and polypeptide chains at the elongation step. The resulting nascent products of both transcription and translation are unstable, whereas completed RNA molecules are partially stabilized in the presence of the drug. (Author abstract) (20 Refs)

14. MORPHOLOGICAL FEATURES OF RECTAL ADENOCARCINOMA FOLLOWING IRRADIATION FROM 25 MEV BETATRON.

Torgushina NS, Iakhontov NE, Obukhova SO
Medical Inst., Gorky, USSR
Vopr Onkol; 28(4):25-29 1982

The effect of brehmstrahlung irradiation from a 25 MEV betatron on rectal adenocarcinoma was studied in 34 patients in comparison with control material from 61 patients. It was found that pronounced changes in tumor tissue are caused by such preoperative treatment: tumor is reduced in size, DNA synthesis and mitotic activity are suppressed considerably and pathological mitoses increase. Morphological observations show that rectal adenocarcinoma is markedly susceptible to brehmstrahlung irradiation from a 25 MEV betatron. (Author abstract) (10 Refs) (In RUS)

CHARACTERIZATION OF THE ENZYMES INVOLVED IN DNA SYNTHESIS, MODIFICATION OR DEGRADATION

15. GENETIC MECHANISMS OF RESISTANCE OF ACYCLOVIR IN HERPES SIMPLEX VIRUS.

Crumpacker CS, Schnipper LE, Chartrand P, Knopf KW
Div. Infectious Disease, Beth Israel Hosp., Room L201, 330 Brookline Ave., Boston, MA, 02215
Am J Med; 73(1A):361-368 1982

Resistance to acyclovir in herpes simplex virus (HSV) is mediated by two genetic loci on the herpes virus genome: viral thymidine kinase and DNA polymerase. Passage of HSV in the presence of 10 uM or 2 uM acyclovir readily coselects for virus deficient in the expression of viral thymidine kinase and resistance to acyclovir. Other workers have shown that passage of HSV in serum-starved cells in the presence of acyclovir selects for a mutant virus possessing a thymidine kinase with an altered substrate specificity and inability to phosphorylate acyclovir. Mutants with temperature-sensitive lesions in the viral DNA polymerase loci of HSV also exhibit resistance to antiviral drug phosphonoacetic acid (PAA) and acyclovir. The mutations conferring resistance to PAA and acyclovir are closely linked within the DNA polymerase locus. The map limits containing mutations that confer resistance to PAA and acyclovir are found within a 1.3 kilobase pair (kbp) region of DNA sequences in HSV-2 (map unit 40.2 to 41.0) and within a 2.6 kbp region of DNA sequence in HSV-1 (map units 40.2 to 41.8). The DNA polymerase loci of HSV also contains a region of DNA sequences to the left of the region defining the resistance mutation which determines the type specific sensitivity of HSV-1 and HSV-2 to acyclovir. Two HSV-1/HSV-2 recombinants were selected for further analysis of resistance. Viral DNA polymerase enzymes were purified from the R6-26 and R6-34 recombinants; the resistant recombinant R6-34 was shown to process an altered viral DNA polymerase. This altered viral DNA polymerase exhibited resistance to three inhibitors of DNA polymerase activity, PAA, acyclovir-TP, and 2'3'dideoxynucleotide triphosphate (didNTPs). The polymerase

from R6-34 and the resistant parent possess a much higher Ki for the PAA and the didNTP than do the sensitive strains. A 1.3 kbp region of HSV-1 DNA within the DNA polymerase locus contains resistance mutations to three DNA polymerase inhibitors, codes for an amino acid sequence of 42,000 mol wt, and defines an active center of the HSV DNA polymerase enzymes. (Author abstract) (32 Refs)

16. HERPES VIRUS SPECIFIC ENZYMES: PROPERTIES, PHYSIOLOGICAL ROLES, AND PHARMACOLOGICAL IMPLICATIONS.

Cheng YC, Nakayama K, Derse D, Bastow K, Ruth J, Tan RS, Dutschman G, Caradonna SJ, Grill S
Dept. Pharmacology, Sch. Medicine, Univ. North Carolina, Faculty Lab. Office Building 231-H, Chapel Hill, NC, 27514
Excerpta Med Int Congr Ser; 571:47-56 1982

After infection of cells with various types of herpes virus, a number of new polypeptides are synthesized, a few of which exhibit specific enzyme activity. A number of enzymes (thymidine kinase, DNA polymerase, DNase, ribonucleotide reductase, deoxyuridine triphosphatase, and uracil-DNA-glycosylase) are discussed in terms of their role as potential targets for selective antiviral compounds. (79 Refs)

17. MECHANISM OF ACTION AND SELECTIVITY OF ACYCLOVIR.

Elion GB
Wellcome Res. Lab., 3030 Cornwallis Road, Research Triangle Park, NC, 27709
Am J Med; 73(1A):7-13 1982

Acyclovir, an acyclic purine nucleoside analog, is a highly potent inhibitor of herpes simplex virus (HSV), Types 1 and 2, and varicella zoster virus, and has extremely low toxicity for the normal host cells. This selectivity is due to the ability of these viruses to code for a viral thymidine kinase capable of phosphorylating acyclovir to a monophosphate; this capability is essentially absent in uninfected cells. The acyclovir monophosphate (acyclo-GMP) is subsequently converted to acyclovir triphosphate (acyclo-GTP) by cellular enzymes. Acyclo-GTP persists in HSV-infected cells for many hr after acyclovir is removed from the medium. The amounts of acyclo-GTP formed in HSV-infected cells are 40 to 100x greater than in uninfected Vero cells. Acyclo-GTP acts as a more potent inhibitor of the viral DNA polymerases than of the cellular polymerases. The DNA polymerases of HSV-1 and HSV-2 also use acyclo-GTP as a substrate and incorporate acyclo-GMP into the DNA primer-template to a much greater extent than do the cellular enzymes. The viral DNA polymerase binds strongly to the acyclo-GMP-terminated template, and is thereby inactivated. (Author abstract) (14 Refs)

18. REPLICATION OF EPSTEIN-BARR VIRUS DNA IN LYMPHOBLASTOID CELLS TREATED FOR EXTENDED PERIODS WITH ACYCLOVIR.

Colby BM, Shaw JE, Datta AK, Pagano JS
Cancer Res. Center, Univ. North Carolina, Box 3, Swing Building, 217H, Chapel Hill, NC, 27514
Am J Med; 73(1A):77-81 1982

An Epstein-Barr virus (EBV)-producing human lymphoblastoid cell line (P3HR-1) was characterized after prolonged exposure to 9-(2-hydroxyethoxymethyl)guanine (acyclovir). P3HR-1 cells grown for 11 mo in the presence of acyclovir had the same number of EBV genomes (approx 15/cell) as did short-term acyclovir-treated cells. After removal of the drug, the number of EBV genome equivalents of long-term acyclovir-treated cultures (15/cell) returned to P3HR-1 control levels (340/cell). Indirect immunofluorescence studies demonstrated reduced percentages of long-term (6 to 12 mo) acyclovir-treated P3HR-1 cells expressing both EBV early antigens (EA) and viral capsid antigens (VCA). This contrasts with short-term acyclovir-treated cultures where EA is expressed at normal levels even though VCA expression is reduced. Restriction fragment patterns of the total EBV DNA from both short- and long-term acyclovir-treated cultures were identical with the exception of two fragments present in short- but not long-term acyclovir-treated cultures. EBV-associated DNA polymerase was present in long-term acyclovir-treated cultures with the level of activity, as determined by assay in vitro, comparable to that in untreated P3HR-1 cells. The polymerase activity in the treated cells was sensitive in vitro to acyclovir triphosphate, N-ethylmaleimide (NEM), and phosphonoacetic acid (PAA). These studies demonstrate that acyclovir inhibits the EBV-productive cycle in P3HR-1 cells continuously during extended periods of treatment. (Author abstract) (21 Refs)

19. A PRELIMINARY STUDY ON THE ANTI-EBV-SPECIFIC DNASE ACTIVITIES OF NASOPHARYNGEAL CARCINOMA (NPC) PATIENTS' SERA.

Pan Q, Huang D, Luo W, Jiao X, Chen J, Chen L
Cancer Inst., Zhogshan Medical Coll., Dongfeng Road IV, Guangzhou, People's Republic of China
Excerpta Med Int Congr Ser; 571:418-422 1982

An anti-DNase activity was demonstrated in the sera of patients in various stages of nasopharyngeal cancer (NPC). A combination of n-butyric acid (4 milliM) and croton oil (400 nanog/ml) was used to induce Epstein-Barr virus (EBV)-specific DNase in EBV-genome-containing cell lines Raji and PeHR-1. An assay utilizing 14C-DNA was devised to determine the effect of NPC patients' sera on the DNase activity of the cell homogenate. Because of the presence of an antibody in the sera of NPC patients, and the neutralizing effect of this antibody to the enzyme, addition of patients' sera markedly decreased the DNase activities to give anti-enzyme rates of 52-55%. The level of serum anti-enzyme activity varied from NPC patient to patient. There appeared to be a good parallelism between the anti-enzyme activity of the serum and its titer to viral capsid antigen to IgA. Further studies are in progress to confirm the practical value of this enzymological test for early detection of NPC. (5 Refs)

20. DNASE II ACTIVITY IN GUERIN ASCITES TUMOUR CELLS.

Avramova ZV, Dessev GN
Inst. Molecular Biology, Bulgarian Acad. Sciences, Sofia, Bulgaria
Dokl Bolg Akad Nauk; 35(1):81-84 1982

The Mg++-Ca++-independent nuclease present in Guerin ascites tumor tissues was studied. The enzyme degraded chromatin by an endonucleolytic mechanism. Results showed that the enzyme was indistinguishable from DNase II, since it was active in the presence of EDTA, showed optimum activity in the acid pH region, had an endonucleotic mode of cleavage with either free or chromatin DNA, yielding 3'-phosphate-containing products, and, on DEAE- or CM-cellulose exhibited a chromatographic behavior similar to that of DNase II. (7 Refs)

21. PURIFICATION OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE OF 60,000 DALTON FROM MAMMALIAN THYMUS AND THYMOMA BY IMMUNOADSORBENT COLUMN AND COMPARISON OF PEPTIDE STRUCTURES.

Nakamura H, Tanabe K, Yoshida S, Matsuyama M, Morita T
Dept. Experimental Radiology, Aichi Cancer Center Res. Inst., Chikusa-ku, Nagoya 464, Japan
Adv Exp Med Biol; 145:19-23 1982

Immunoadsorbent column chromatography, which is suitable to purify terminal deoxynucleotidyl transferase (TdT), was developed to study the differences in the mol wt of TdT and investigate whether the differences reflect species- or tissue-specificities of the enzyme, or are due simply to the proteolytic degradation during purification process. Results suggested that a single polypeptide with 60,000 dalton (60-k) must be the original form of mammalian TdT, and the alpha and beta subunits of the enzyme might be proteolytic products derived from the 60-k form. Tryptic peptide maps suggested homology in amino acid sequences among the TdT's from three mammalian species. (10 Refs)

22. ENRICHMENT OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE ACTIVITY BY CELL SEPARATION.

Sarin PS, Virmani M, Gallo RC
Lab. Tumor Cell Biology, NCI, Bethesda, MD, 20205
Adv Exp Med Biol; 145:337-356 1982

The technique of free flow electrophoresis was utilized for separation of human leukemic cells into terminal deoxynucleotidyl transferase (TdT) positive cell populations. With the technique, an up to 20-fold enrichment in TdT positive cells was obtained thus making the detection of low levels of enzyme simpler, and providing a tool for selection and identification of cell types containing the enzyme for biochemical and biological studies. With the use of the technique enrichment of TdT positive cell populations from normal human bone marrow, cells from patients with acute lymphoblastic leukemia, and chronic myelogenous leukemia in blast phase of the disease were enriched. (27 Refs)

23. ENZYMATIC PROPERTIES IN PERICELLULAR MEMBRANES OF LEUKEMIC CELLS.

Losa G
Lab. Cellular Pathology, Ticino Inst. Pathology, Locarno, Switzerland
Adv Exp Med Biol; 145:377-384 1982

Enzyme characteristics of the plasma membrane were analyzed biochemically in cell populations isolated from the peripheral blood of leukemic patients to distinguish the various types of acute leukemias. Very low enzyme activities were generally recorded in cells of both non B/non T lymphoblastic and T leukemias. Differences in the activity of some enzymes were sufficient to allow a clear-cut differentiation of the two leukemic cell populations. In lymphoblastic leukemia high 5'-nucleotidase activity was measured with a mean level of approx 45 nanomole (nmol)/hr/10(6) cells. In some patients, the activity reached a mean value of 230 nmol/hr/10(6) cells, suggesting an earlier differentiation stage of the corresponding leukemic cells. Compared to the control mononuclear cell population, moderate decreased activities were measured for 5'-nucleotidase, alkaline phosphomonoesterase, and gamma-glutamyltranspeptidase. The activity of 5'-phosphodiesterase was significantly high in approx 40% of the patients while in the remainder it was practically undetectable or very low (range 0-2.5 nmol/hr/10(6) cells). The absence of activity of 5'-phosphodiesterase was inversely related to the expression of the immune associated antigen (Ia), whereas the absence of Ia correlated with the presence of high activity (range 3.8-16.8 nmol/hr/10(6) cells). A positive low level of terminal deoxynucleotidyl transferase activity was observed in approx 20% of patients but with an activity range (0-30 picomole/min/mg protein) and distribution similar for both subgroups of acute myeloid leukemia. (14 Refs)

24. PURIFICATION AND CHARACTERIZATION OF MULTIPLE FORMS OF TERMINAL TRANSFERASE FROM HUMAN LEUKEMIC CELLS.

Deibel MR, Coleman MS, Hutton JJ
Dept. Biochemistry, Univ. Kentucky, Lexington, KY
Adv Exp Med Biol; 145:37-60 1982

Biochemical properties of human terminal transferase (TdT) were analyzed in human lymphoblasts derived from therapeutic leukapheresis. Isolation of the purified enzyme demonstrated that, regardless of the source or mol wt, the protein is catalytically active and appears to be homogeneous in structure. Determination of the kinetic mechanism provided a model system for the comparable, yet far more complex eucaryotic DNA polymerases. The reaction catalyzed by human TdT followed a rapid equilibrium random mechanism as determined by initial velocity and product inhibition studies with oligodeoxyadenylate initiators. (15 Refs)

25. USE OF IMMUNOFLUORESCENCE TESTS FOR TDT IN HUMAN HEMATOLOGIC MALIGNANCY.

Coleman MS, Hutton JJ
Dept. Biochemistry, Univ. Kentucky, Lexington, KY
Adv Exp Med Biol; 145:109-114 1982

In clinical samples (bone marrow, peripheral blood, testicular tissue, cerebrospinal fluid, and lymph nodes) terminal deoxynucleotidyl transferase (tdT) was detected by a quantitative radiometric assay and by an immunofluorescence assay. The quantitative assay utilized a homopolymer of av chain length (50 residues) and deoxy guanosine triphosphate. Both rabbit anti-calf serum and rabbit anti-human serum gave nearly identical results. An analysis of individual samples grouped by disease showed that no systematic error exists in detecting TdT protein. Shipment of samples significantly reduced positive immunofluorescence. (5 Refs)

26. PROGNOSTIC SIGNIFICANCE OF TERMINAL TRANSFERASE ACTIVITY AND OTHER FACTORS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA.

Hutton JJ, Coleman MS, Moffitt S
Dept. Medicine, Univ. Texas, San Antonio, TX
Adv Exp Med Biol; 145:243-257 1982

Activity of terminal deoxynucleotidyl transferase (TdT) was measured in cells from the bone marrow and peripheral blood of 206 children (less than 1-16 yr old, av 77 mo) with acute lymphoblastic leukemia (ALL) to investigate the prognostic significance of the quantitative variations in level of TdT at diagnosis and to find out whether quantitative measurements of TdT activity during remission of leukemia can predict relapse of disease. Complete remission (CR) was obtained in 96% of patients, and 43% relapsed during the course of the study. The median duration of CR was 1,400 days. Maintenance chemotherapy was generally continued for 3 yr after complete remis-

sion was achieved or until relapse occurred. The mean TDT activity in the bone marrow of 157 patients was 91 +-143 units (U)/10(8) cells. For peripheral blood the mean activity was 54 +-159 U/10(8) cells of 163 specimens. The large standard deviation reflects the wide range of activities seen in patients with ALL. A significant proportion of the variation was due to a relatively small number of patients with extremely high activities. The bone marrow from 198 children without ALL contained 5.9 U of TdT activity/10(8) cells with a standard deviation of TdT measurements could not be used to predict malignancy because significant overlap of the two groups was observed. Results showed that the quantitative activity of TdT in blasts at diagnosis of children with typical ALL does not convey prognostic information. (5 Refs)

27. THE PROGNOSTIC SIGNIFICANCE OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE (TDT) IN PATIENTS WITH LEUKEMIAS AND MALIGNANT LYMPHOMAS.

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Adv Exp Med Biol; 145:259-277 1982

Activity of terminal deoxynucleotidyl transferase (TdT) was analyzed and the relationship to remission frequency and duration as well as survival in patients with acute lymphoblastic leukemia (ALL), acute nonlymphoblastic leukemia (ANLL), and malignant non-Hodgkin's lymphoma (NHL) in adults was investigated. All patients were diagnosed and treated according to standardized chemotherapy protocols. Of 113 patients treated on ALL protocols including 101 patients with ALL and 12 patients with lymphoblastic lymphoma, 44 patients were studied for TdT activity at diagnosis prior to treatment and an additional 38 patients after initiation of treatment or at relapse. Of 72 patients with evaluable TdT determinations, only 2 with B cell ALL and 1 with T cell ALL failed to exhibit elevated TdT activities. Pre-treatment bone marrow TdT activities were found to be significantly related to survival: patients exhibiting TdT activities less than 1 unit (U)/10(8) cells showed the longest survival. Thirteen of 91 patients with ANLL receiving chemotherapy exhibited elevated TdT activities as observed in ALL and 11 (12%) exhibited low (less than 0.1 U/10(8) cells) but unequivocal TdT activity. Of 52 patients with diffuse NHL treated with protocol chemotherapy, 50% of null cell lymphomas and 52% of T cell lymphomas exhibited high levels of TDT activity. These findings documented the prognostic significance regarding survival of levels of TDT activity in ALL, ANLL, and diffuse NHL. (32 Refs)

28. CLINICAL RELEVANCE OF TERMINAL TRANSFERASE AD ADENOSINE DEAMINASE IN LEUKEMIA.

Brusamolino E, Bertazzoni U, Isernia P, Ginelli E, Scovassi AI, Zurlo MG, Plevani P, Sacchi N, Bernasconi C
Divisione di Ematologia, Policlinico S. Matteo, Pavia, Italy
Adv Exp Med Biol; 145:279-303 1982

Correlation between terminal deoxynucleotidyl transferase (TdT) and adenosine deaminase (ADA) was investigated in 119 adults and 63 children with acute leukemia at presentation, 40 patients with acute lymphoblastic leukemia (ALL) at clinical relapse, 90 patients with acute leukemia in clinical remission, 42 adults with Philadelphia chromosome-positive (Ph'+) chronic myelocyte leukemia (CML) in chronic phase, 28 adults with Ph'+ CML in blastic phase, 3 patients with Ph'+ acute leukemia presenting as ALL, and 15 non-neoplastic human controls. All seven T-ALL adults were positive for TdT, whereas the three B-ALL adults were TdT negative; 26/28 non-T, non-B ALL patients were TdT positive. In children, TdT was positive in 8/9 patients with T-ALL and in 35/41 with non-T, non-B ALL; the single B-ALL patient was TdT negative. The ADA enzymatic assay was performed in 27 adults and 9 children with ALL. The range of ADA varied between 20-7,580 units (U)/10(8) cells, with a mean of 1,458 +-393 U/10(8) cells in adults and 745 +-334 U/10(8) cells in children; a mean of 113 +-16 was found in non-neoplastic controls. The TdT enzymatic assay was strictly negative in the peripheral blood of 18 Ph'+ leukemia patients. The av value of ADA activity in Ph'+ leukemias in stable phase (25 patients) was 103 +-15 U/10(8) cells, which was very close to that obtained for 10 non-neoplastic controls. The values of ADA were increased over the controls only in two patients showing early signs of transformation. (28 Refs)

29. STUDIES OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE IN NORMAL AND NEOPLASTIC HUMAN CELLS.

Campagnari F, Clerici L, Bombardieri E, Vezzoni P, Di Fronzo G, Villa ML, Buraggi GL
Lab. Biochemistry, Biology Group D.G. XII, C.E.C. Joint Res. Centre, 21020 Ispra (Va), Italy
Adv Exp Med Biol; 145:305-320 1982

Procedures for measurements of terminal deoxynucleotidyl transferase (TdT) activity and the immunofluorescence method for cells containing TdT were standardized and were used in analysis of 36 leukemia and 75 lymphoma patients not receiving chemotherapy. TdT was almost undetectable in the preparations from WBC of chronic lymphocytic leukemias and from 12 normal lymph nodes. The enzyme levels were well below the values of 10 units (U)/10(8) cells or 10 U/0.1 g lymphoid tissue, that were established as limits for discriminating between the trivial and the pathologically significant findings. Moreover, the cells from both sources did not fluoresce in the immunoassay for TdT. TdT was elevated in the neoplastic cells from 16/19 patients with acute lymphoblastic leukemia (ALL), and was practically undetectable only in 2 patients with common ALL and the single patient with B ALL. In the group of eight patients with common ALL whose blasts were analyzed biochemically and were found to contain appreciable amounts of enzyme, the concentration of TdT/10(8) cells ranged from 78-648 U with a mean value of 244 U. TdT was found in all 10 patients with lymphoblastic lymphoma, concentrations ranging from 289-1,301 U/0.1 g tissue with a mean of 630 U. In most malignant lymphoblasts the TdT immunofluorescence was restricted to the nuclei. Definite correlations between concentrations of enzymatic activity and percentage of immunofluorescent cells could not be established. (33 Refs)

30. TDT-POSITIVE AND TDT-NEGATIVE HUMAN LEUKEMIC CELLS: SPECIFIC DENSITY AND MORPHOLOGY.

Marini M, Bagnara GP, Biagini G, Baccarani M, Rosito P
Istituto di Istologia ed Embriologia Generale, Facolta di Medicina, 401000 Bologna, Italy
Adv Exp Med Biol; 145:357-370 1982

Cell density distribution of normal terminal deoxynucleotidyl transferase (TdT) positive bone marrow cells was compared with that of TdT positive leukemic blast cells to study the nature of the leukemic populations. Ultrastructural features of cell fractions expressing or not expressing the marker in the

different leukemias were examined. In 10 untreated patients with Philadelphia chromosome (Ph1) positive chronic myeloid leukemia (CML) studied at the onset of the disease, no TdT activity was observed. One of the patients underwent a TdT positive blastic crisis 8 mo later, suggesting that TdT positive cells arise, at least in a sizable number at the onset of the blastic crisis and then accumulate rapidly. TdT was evaluated in 31 patients during the blastic crisis of CML and in 3 patients undergoing the blastic transformation following other myeloproliferative disorders. Fourteen CML-blastic crisis TdT positive cells were found in 14 CML-blastic crisis patients, the involved lymph nodes in one additional patient, and in the lymph nodes of a patient with agnogenic myeloid metaplasia in blastic proliferation. Three TdT positive and two TdT negative patients were Ph1 negative. No correlation between TdT-positivity and length of chronic phase was observed. A total of 67 patients with acute lymphoid leukemia were studied during a 3-yr period. Nine of 10 TdT negative adult patients had very low peripheral blast cell counts (less than 500/ul), which might have been caused by the insensitivity of the assay for very small numbers of TdT positive cells. (17 Refs)

31. NUCLEAR TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE (TDT) IN LEUKEMIC INFILTRATES OF TESTICULAR TISSUE.

Janossy G, Thomas JA, Eden OB, Bollum FJ
Dept. Immunology, Royal Free Hosp., London, England
Adv Exp Med Biol; 145:321-330 1982

Indirect immunofluorescence (IF) and immunoperoxidase (IP) techniques were used to determine the optimal method for identifying terminal deoxynucleotidyl transferase (TdT) positive leukemic blasts in 40 testicular biopsies. In 11 patients the routine histology of testicular biopsies (taken as routine procedure or for clinical suspicion) showed heavy leukemic infiltrates. In nine of these patients strong nuclear TdT staining was observed in the infiltrating lymphoblasts while in two patients cells were present but were TdT negative. In some patients the leukemic blasts were heterogeneous and some contained no identifiable TdT enzyme in the vicinity of strongly positive blasts. The TdT positivity corresponded to the nucleus and clear cytoplasmic staining was observed only in the dividing cells where TdT is known to be released from the disrupted nucleus. In seven biopsies from patients with treated acute lymphoblastic leukemia (ALL) the normal structure of seminiferous tubules was preserved and no histological evidence of increased lymphoid or lymphoblastic infiltration was observed. Large areas of the tissue were void of TdT positive cells. No identifiable TdT positive cells were present in another seven testicular biopsies from patients with treated ALL. With one exception all patients were regarded at the histological investigation as apparently non-involved samples. (14 Refs)

32. THE BOLLUM ENZYME IN LEUKEMIA AND LYMPHOMA CELLS: THE FIRST DECADE.

McCaffrey R, Bell R, Lillquist A, Wright G, Baril E
Section Medical Oncology, Evans Memorial Dept. Clinical Res., Boston Univ. Medical Center, Boston, MA
Adv Exp Med Biol; 145:221-241 1982

The conceptional and therapeutic significance of terminal deoxynucleotidyl transferase (TdT) expression in malignant hemopoietic cells is reviewed. Some preliminary data on the potential utility of 6-anilinouracils as specific cytocidal agents for TdT-positive leukemias are also included. Survey studies of the expression of TdT in malignant cells from a wide spectrum of hemopoietic neoplastic processes have been performed and the data on the first several hundred patients surveyed are presented. A total of 30 patients with Philadelphia-chromosome positive blastic chronic myeloid leukemia was studied in a multiinstitutional cooperative clinical trial. Only 1/14 TdT-negative patients responded to vincristine-prednisone therapy, whereas 11/16 TdT-positive cases achieved remission. TdT heterogeneity at the molecular level occurs both as a proteolytic artefact during purification and as a result of metabolic modification (phosphorylation-dephosphorylation). The occurrence of TdT positivity in leukemia cells with myeloid characteristics suggests that TdT may be present in an early stem cell compartment. Effect of a large series of uracil analogues on human leukemic and calf thymus TdT was analyzed. Two compounds showed significant and specific inhibition. The analysis of molecular forms of TdT has not yet yielded a means to discriminate between normal and malignant TdT bearing populations. (44 Refs)

MECHANISMS OF DNA REPAIR

33. NATURE OF THE CONSTITUTIVE AND INDUCED MAMMALIAN O6-METHYLGUANINE DNA REPAIR ENZYME.

Craddock VM, Henderson AR, Gash S
MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey, England
Biochem Biophys Res Commun; 107(2):546-553 1982

Previous studies indicate that removal of O6-methylguanine (O6-MG) from DNA occurs by transfer of the methyl group from the O6-position of guanine to a cysteine residue in protein, forming acid-stable S-methylcysteine (S-MC). However, there is evidence that the reaction product is acid labile. This inconsistency was investigated. The amount of O6-MG removed from DNA by extracts or rat liver and Raji cells in vitro was compared with the amount of S-MC formed. The O6-MG DNA repair enzyme was extracted from rat liver as previously described. The DNA used as substrate was methylated with [3H-methyl]nitroso-methylurea. The enzyme concentration curve showed that the amount of S-MC formed was proportional to the amount of enzyme added. The nature of the increased ability of liver of diethylnitrosamine (DEN)-treated rats to remove O6-MG was also investigated by study of enzyme activity of liver extracts. DEN treatment increased repair enzyme activity approx 3x. The data supports the view that removal of O6-MG from DNA occurs by transmethylation, the methyl group giving rise to acid-stable S-MC residues in protein. The induced enzyme, like the constitutive enzyme, appears to be a transmethylase. (26 Refs)

34. REPAIR RESPONSES TO DNA DAMAGE: ENZYMATIC PATHWAYS IN E COLI AND HUMAN CELLS.

Hanawalt PC, Cooper PK, Ganesan AK, Lloyd RS, Smith CA, Zolan ME
Dept. Biological Sciences, Stanford Univ., Stanford, CA, 94305
J Cell Biochem; 18(3):271-283 1982

Known responses to damaged DNA in bacteria and mammalian systems are reviewed and recent approaches to the study of DNA repair is described. Topics discussed include constitutive and inducible repair of DNA, the role of chromatin struc-

ture in DNA repair, and activities of endonuclease V of bacteriophage T4 and properties of the cloned denV gene. (66 Refs)

35. BIOPSY MEASUREMENT OF DNA DAMAGE AND REPAIR IN VIVO: SINGLE-STRAND BREAKS, ALKALI-LABILE BONDS, AND ENDONUCLEASE-SENSITIVE SITES.

Brash DE, Hart RW
Sidney Farber Cancer Inst., 44 Binney St., Boston, MA, 02115
Radiat Res; 91(1):169-180 1982

Measurement of DNA single-strand breaks plus alkali labile bonds by alkaline sucrose density gradient centrifugation was adapted to in vivo biopsy samples by refinement of a sensitive fluorescence assay to detect nanogram quantities of nonradiolabeled single-stranded DNA in high-salt alkaline sucrose. The DNA concentration required is low enough to avoid artifactually rapid sedimentation of aggregated single strands and DNA-membrane complexes and to enable analysis of 8 ul biopsy samples. The fluorescent reagent is 3,5-diaminobenzoic acid-2HCl (DABA). Female Sprague-Dawley rats were subjected to x-irradiation and liver biopsies were analyzed by the new method; UV-irradiated rat liver nuclei were also studied after exposure to increasing concentrations of endonuclease. DABA reacted equally with single- and double-stranded DNA, and thus is not preferential for rapidly renaturing reiterated sequences. The nuclei isolation procedure liberated nearly all nuclei, and the squash procedure did not introduce strand breakage. The present method is suitable for smaller samples and less invasive techniques, such as fine-needle biopsy. It eliminates the problems of aggregated sedimentation and DNA damage by incorporated radiolabel, making it suitable for monitoring the role of DNA alterations of pathogenic processes in vivo. (52 Refs)

36. COMPARISON OF NUCLEOID AND ALKALINE SUCROSE GRADIENTS IN THE ANALYSIS OF INHIBITORS OF DNA REPAIR IN HUMAN FIBROBLASTS.

Charles WC, Cleaver JE
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Biochem Biophys Res Commun; 107(1):250-257 1982

Effects of the DNA repair inhibitors hydroxyurea (HU), aphidicolin (APH), cytosine arabinoside (ARAC), dideoxythymidine (dDT), and 3-aminobenzamide (AB) on nucleoid and alkaline sucrose sedimentation patterns of DNA from normal human fibroblasts (2498, BD, ANA-1) or embryonic cells (MRC-5) following UV-irradiation were investigated. Single-strand breaks produced by inhibition of DNA repair by APH maintained complete relaxation of DNA during nucleoid sedimentation, but those produced by ARAC or dDT did not. Skin and embryonic fibroblasts differed in the rate at which their nucleoids recovered rapid sedimentation rates after irradiation, despite similarities in repair. Embryonic cells had a more rapid growth rate and a larger fraction of cells in DNA synthesis than the skin fibroblasts. These differences may have modified the changes in sedimentation rate that are due to excision repair itself. The absence of any effect of AB on nucleoid sedimentation indicated that poly(ADP-ribose) synthesis was not involved in the repair of UV damage in human fibroblasts. Results indicated that the two kinds of gradients employed here cannot be used interchangeably, but can provide useful insights into the intermediate stages of DNA repair in comparative studies. (19 Refs)

37. METHODS FOR THE DETECTION OF SINGLE-STRAND BREAKS IN DNA UNDER NEUTRAL CONDITIONS AND THEIR APPLICATION IN A STUDY ON THE MECHANISM OF REPAIR OF N-METHYLATED PURINES IN MOUSE CELLS.

Wintersberger E
Institut fur Molekularbiologie der Universitat Wein, Wasagasse 9, A-1090 Wien, Austria
Eur J Biochem; 125(1):151-156 1982

Considering enzymatic activities found in bacteria and in animal cells, there are two possible mechanisms for repair of N-methylated purines produced by methylating agents such as the mutagen and carcinogen N-methyl-N'-nitro-N-nitrosoguanidine. Both mechanisms involve first an enzymatic removal of the methylated bases by glycosylases. The resulting apurinic sites could then be repaired by (a) direct insertion of the correct bases purine insertases or (b) opening of the polynucleotide chain by apurinic endonuclease followed by repair synthesis. As the methods commonly used to detect lesions induced by methylating agents involve alkali, it was thus far not possible to decide between the above possibilities because apurinic sites are by themselves alkali labile. In this paper I describe two methods which avoid alkali and therefore allow the clarification of some aspects of the repair reaction. One of these methods makes use of 95% formamide at 40 C in place of alkali to denature DNA with pre-existing single-strand breaks, the other measures the capacity of DNA scissions with free 3'-OH groups to act as primer for Escherichia coli DNA polymerase I. Results obtained with both methods make it unlikely that purine insertases play a major role in the repair of apurinic sites. Kinetics of production and repair of single-strand breaks, produced in 3T6 mouse fibroblasts by incubation with N-methyl-N'-nitro-N-nitrosoguanidine, were also examined using the methods of alkaline elution and alkaline sucrose gradient centrifugation. (Author abstract) (29 Refs)

38. EFFECT OF HYPERTHERMIA AT 42 AND 45 C ON REPAIR OF RADIATION-INDUCED DNA STRAND BREAKS IN CHO CELLS.

Dikomey E
Inst. Biophysics and Radiobiology, Univ. Hamburg, Martinistrasse 52, D-2000 Hamburg 20, W. Germany
Int J Radiat Biol; 41(6):603-614 1982

The effect of hyperthermia on DNA strand break repair was studied in CHO cells. DNA strand breaks were analyzed by the alkaline DNA-unwinding technique followed by chromatography on hydroxyapatite. Immediately after irradiation with doses ranging from 2 to 7 Gy, cells were exposed to 42 or 45 C. Heat alone was found to induce DNA strand breaks only at temperatures exceeding 45 C. In comparison to 37 C, the rate of single-strand break repair was increased by hyperthermia at 42 C, but decreased at 45 C. In contrast hyperthermia at either temperature resulted in a higher number of remaining double-strand breaks 1 hr after irradiation. For the three treatments applied, ie x-rays alone or combined with hyperthermia at 42 or 45 C, the relation between cell survival and the number of double-strand breaks measured 1 hr after irradiation could be described by the same function. (Author abstract) (42 Refs)

39. DNA DOUBLE-STRAND BREAKS GENERATED BY THE REPAIR OF X-RAY DAMAGE IN CHINESE HAMSTER CELLS.

Ahnstrom G, Bryant PE
Wellenberg Lab., Univ. Stockholm, S-106 91 Stockholm, Sweden
Int J Radiat Biol; 41(6):671-676 1982

X-irradiated Chinese hamster cells were treated with a mixture of detergent (to permeabilize cells) and enzymes derived from Micrococcus luteus immediately after irradiation to reveal possible double strand breaks (dsb). In a second experiment cells were incubated after irradiation allowing time for cellular enzymes to recognize double strand damaged sites and induce dsb. Both treatments resulted in an increased yield of dsb, indicating that double strand lesions of the types, single strand break-endonuclease sensitive (es) or es-es, occur in the DNA and that the lesions can be converted into dsb during postoperative incubation. (16 Refs)

40. DNA-REPAIR SYNTHESIS IN BLAST CELLS FROM PATIENTS WITH ACUTE MYELOBLASTIC LEUKAEMIA.

Lewensohn R, Ringborg U
Radiumhemmet, Karolinska Hosp., S-401 01 Stockholm, Sweden
Scand J Haematol; 28(5):399-407 1982

DNA-repair synthesis was studied in blast cells from patients with acute myeloblastic leukemia. The DNA-repair synthesis, measured as 3H-thymidine incorporation, was registered as unscheduled DNA synthesis by autoradiography, and also after suppression of the replicative DNA synthesis by hydroxyurea by liquid scintillation. There was good correlation when results from both methods were compared. A significantly wider variation of unscheduled DNA synthesis was found in leukemic as compared to normal blast cell populations. There was also a wider variation within individual leukemic blast cell samples compared to normal samples. A correlation was found between unscheduled DNA synthesis induced by nitrogen mustard and by UV-irradiation in the leukemic blast cell populations. The UV-induced unscheduled DNA synthesis in some of the leukemic blast cell populations continued to increase at doses of UV-irradiation at which normal myeloblasts had reached their max level of unscheduled DNA synthesis. This indicates that at least some malignant blast cell populations may possess an effective DNA-repair system. (Author abstract) (21 Refs)

41. ABNORMAL SENSITIVITY TO UV-RADIATION IN CULTURED SKIN FIBROBLASTS FROM PATIENTS WITH HEREDITARY CUTANEOUS MALIGNANT MELANOMA AND DYSPLASTIC NEVUS SYNDROME.

Smith PJ, Greene MH, Devlin DA, McKeen EA, Paterson MC
MRC Clinical Oncology Radiotherapeutic Unit, The Medical School, Hills Road, Cambridge CB2 2QH, England
Int J Cancer; 30(1):39-45 1982

The dysplastic nevus syndrome (DNS) is a preneoplastic melanocyte abnormality which occurs in families affected by hereditary cutaneous malignant melanoma (HCMM). Although environmental exposures, especially solar UV-irradiation, have been implicated as risk factors in sporadic melanoma, the role of such exposures in the pathogenesis of HCMM is unknown. We have studied the in vitro radiation responses of six nontumor skin fibroblast strains from HCMM/DNS patients representing five families. All six HCMM/DNS strains were found to show some degree of enhanced cell killing sensitivity, compared with normal controls, following 254 nm UV-irradiation. The abnormal survival responses appeared to relate to specific characteristics of HCMM/DNS cells since the six strains had essentially normal sensitivity to gamma-radiation. The enhanced photosensitivity was not associated with abnormal patterns in either DNA repair synthesis or UV-induced inhibition and recovery of de novo DNA synthesis. The survival results are consistent with the hypothesis that the genetically determined predisposition to malignant melanoma may directly or indirectly be the consequence of increased susceptibility to UV-induced cellular damage. (Author abstract) (38 Refs)

42. ENHANCEMENT OF ULTRAVIOLET-INDUCED UNSCHEDULED DNA SYNTHESIS IN HELA CELL LYSATE BY APHIDICOLIN IN VIVO.

Hanaoka F, Yamada K, Kato H, Yamada M
Dept. Physiological Chemistry, Faculty Pharmaceutical Sciences, Univ. Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan
Biochem Biophys Res Commun; 107(1):1-5 1982

The effect of DNA synthesis inhibitors on UV-induced unscheduled DNA synthesis in HeLa cells and in cell-free homogenates was investigated. Aphidicolin inhibited unscheduled DNA synthesis (UDS) at UV damaged sites in cellular DNA. This inhibition was reversible in cell free homogenates fortified with sufficient deoxynucleotide-triphosphates. A linear and parallel correlation was observed between UDS in intact cells and incorporation of tritiated deoxythymidine triphosphate in homogenates. Combined treatment with aphidicolin and hydroxyurea induced higher UDS in cell-free homogenates than did aphidicolin alone. 2',3'-Dideoxythymidine and arabinofuranoysl cytosine had little effect on UDS. (17 Refs)

43. REPAIR AND MUTAGENESIS OF PLASMID DNA MODIFIED BY ULTRAVIOLET IRRADIATION OR N-ACETOXY-N-2-ACETYLAMINOFLUORENE.

Schmid SE, Daune MP, Fuchs RP
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Proc Natl Acad Sci USA; 79(13):4133-4137 1982

Plasmid DNA was modified in vitro to various extents with N-acetoxy-N-2-acetylaminofluorene or UV irradiation. The modified plasmid DNAs were then used to transform Escherichia coli strains having different repair capabilities. Both survival and mutagenesis frequencies of the plasmid were measured as a function of the number of lesions per plasmid molecule. The majority of N-2-acetylaminofluorene (AAF) adducts, like thymine dimers, were repaired by the excision (uvrA+-dependent) pathway. In rec+ strains, dose-dependent mutagenesis occurred in either AAF- or UV-modified plasmid DNA. This is in contrast with results obtained in recA- strains, in which only AAF adducts gave rise to a lower, but dose-dependent, mutagenesis frequency. In these recA- strains there was no UV mutagenesis. Unlike what is observed with phages, induction of the 'SOS' functions by UV irradiation of the bacteria prior to transformation did not increase the survival or the mutagenesis of the plasmid. (Author abstract) (47 Refs)

44. MUTATIONAL SPECIFICITY OF UV LIGHT IN ESCHERICHIA COLI: INDICATIONS FOR A ROLE OF DNA SECONDARY STRUCTURE.

Todd PA, Glickman BW
(c/o Glickman) Lab. Molecular Genetics, Natl. Inst. Environmental Health Sciences, Research Triangle Park, NC, 27709
Proc Natl Acad Sci USA; 79(13):4123-4127 1982

We used the lacI forward mutagenesis system to determine the mutational specificity of UV-induced mutation in a repair-proficient (Uvr+) and a repair-deficient (deltaUvrB) strain of Escherichia coli. The spectra recovered at similar levels of mutagenesis were similar, the exception being a mutational hotspot at site A24 specific to the deltaUvrB strain. Mutations induced at this hotspot, as well as those induced at other mutational hotspots that were found to be common to both the Uvr+ and Uvr- strains, involve G-C to A-T transitions. All of the hotspots are at sites of potential dipyrimidine photoproducts, such as thymine-cytosine and cytosine-cytosine dimers, or of pyrimidine-cytosine photo-product Py-C* lesions. Each of these hotspots occurs at a site in the potential hairpin loop of quasi-palindromic sequences. These observations suggest an important role for DNA structure in determining the fate of UV-induced premutational lesions. (Author abstract) (38 Refs)

45. METABOLISM OF DITP IN HELA CELL EXTRACTS, INCORPORATION INTO DNA BY ISOLATED NUCLEI AND RELEASE OF HYPOXANTHINE FROM DNA BY A HYPOXANTHINE-DNA GLYCOSYLASE ACTIVITY.

Myrnes B, Guddal PH, Krokan H
Inst. Medical Biology, Univ. Tromso, 9001 Tromso, Norway
Nucleic Acids Res; 10(12):3693-3701 1982

Slow, nonenzymatic hydrolysis causes generation of dITP from dATP. While [3H]dITP was degraded rapidly to [3H]deoxyinosine by HeLa cell nuclear extracts, no net degradation of [3H]dITP was observed in the presence of physiological concentrations of ATP, apparently because the extract contained deoxynucleoside diphosphate kinase activity that regenerated [3H]dITP from [3H]dIDP. Isolated HeLa cell nuclei, as well as partially purified DNA polymerase alpha, incorporated [3H]dITP into DNA at 50-60% of the rate of [3H]dGTP incorporation. No rapid release of the incorporated radioactivity was observed. The mol wt of nascent DNA containing dIMP residues, however, decreased slightly after prolonged incubation in the presence of EDTA, suggesting that a repair process is initiated in dIMP-containing chromatin. Furthermore, release of free[3H]hypoxanthine from [3H]dIMP-containing DNA was detected after incubation with nuclear extracts in the presence of EDTA, suggesting the presence of hypoxanthine-DNA glycosylase activity in HeLa cell nuclei. (Author abstract) (26 Refs)

DNA/ CHROMATIN SEQUENCE, STRUCTURE AND MODIFICATION

46. N-2-ACETYLAMINOFLUORENE MODIFICATION OF POLY(DG-M5DC).POLY(DG-M5DC) INDUCES THE Z-DNA CONFORMATION.

Santella RM, Grunberger D, Nordheim A, Rich A
Cancer Center/Inst. Cancer Res., Columbia Univ. Coll. Physicians and Surgeons, 701 W 168 St., New York, NY, 10032
Biochem Biophys Res Commun; 106(4):1226-1232 1982

Carcinogen induced conformational changes and the effect of N-2-acetylaminofluorene (N-Aco-AAF) modification on poly(dG-m5dC).poly(dG-m5dC) were investigated. The circular dichroism spectra of poly(dG-m5dC).poly(dG--m5dC) with various levels of acetylaminofluorene (AAF) modification are presented. With increasing levels of AAF, a decrease in the positive band at 280 nanometer (nm) and an increase in the negative band at 255 nm was observed. A sample with 3.5% modification underwent the B to Z transition with increasing Mg++ concentrations. A DNA sample modified with [14C] labeled AAF to an extent of 8.5% showed approx 50% digestion with S1 nuclease after 3 hr. In contrast, a 17.5% AAF modified sample of poly(dG-m5dC).poly(dG-m5dC) showed only 14% digestion after 3-hr incubation with S1 nuclease. The equilibrium binding level of the various polymers as a function of the concentration of N-Aco-AAF was determined. At the lowest concentration of N-Aco-AAF added, the methylated polymer was modified to a level of 6% compared to 3% with the unmethylated polymer. At higher concentrations of N-Aco-AAF, the level of AAF binding in the methylated polymer rose more rapidly than in the unmethylated polymer. Thus, the order of reactivity with AAF is poly(dG-m5dC).poly(dG-m5dC) greater than poly(dG-dC).poly(dG-dC) greater than poly(dG).poly(dC). Results indicated that the alternating polymer poly(dG-m5dC).poly(dG-m5dC) undergoes the B to Z transition at lower levels of AAF modification (10%) than required to convert the non methylated polymer (greater than 20%). (18 Refs)

47. SOME EVIDENCE FOR INTERACTION OF D-CYCLOSERINE WITH DNA.

Matsuda Y, Kitahara M, Maeda K, Umezawa H
Inst. Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan
J Antibiot (Tokyo); 35(7):893-899 1982

D-Cycloserine showed a decreased lethality against paraquat-treated E coli than against non-treated cells. E coli K12 mutants which were deficient in DNA repair were more sensitive than the wild type strain to D-cycloserine. Furthermore, D-cycloserine caused PM2 DNA cleavage in the presence of Fe++, and the cleavage was strongly inhibited by singlet oxygen scavengers. These results indicate that D-cycloserine interacts with DNA and an active oxygen species is involved in the interaction. (Author abstract) (14 Refs)

48. RESTRICTION ENZYME CLEAVAGE OF ULTRAVIOLET-DAMAGED DNA.

Cleaver JE, Samson L, Thomas GH
Lab. Radiobiology and Environmental Health, Univ. California, San Francisco, CA, 94720
Biochim Biophys Acta; 697(2):255-258 1982

SV40 and pBR322 DNAs damaged by ultraviolet light were cleaved abnormally by several restriction enzymes because of

damage to pyrimidines in the recognition sequences. The use of a tandemly duplicated plasmid provided a particularly sensitive target molecule for detecting pyrimidine dimers and other possible photoproducts. The relative efficiency with which cleavage was blocked (HindIII greater than TaqI greater than EcoRI greater than BamI greater than SalI much greater than HhaI,HaeIII) corresponds approximately to the relative frequency of pyrimidine dimer formation in the recognition sequences, but at a slightly higher frequency in potential sites for the non-cyclobutane T-C product. The pyrimidine dimers appear to have a range of influence that extends 1 to 3 base-pairs along the DNA molecule. These effects provide clues to the way DNA damage from mutagens and carcinogens can interfere with specific enzyme-DNA interactions. (Author abstract) (21 Refs)

49. NITROSOUREAS: CURRENT STATUS AND NEW DEVELOPMENTS.

Prestayko AW, Baker LH, Rooke ST, Carter SK, Schein PS, Alder NA
Res. and Development, Bristol Laboratories, Syracuse, NY, 13201
Nitrosoureas: Current Status and New Developments. New York, Academic Press, 416 pp., 1981.

The preclinical and clinical status of nitrosoureas (NUA) are reviewed. Preclinical studies involving the development of NUA, combination chemotherapy including NUA against animal tumors, novel NUA, chemical decomposition of chloroethylnitrosoureas, metabolism of NUA, DNA crosslinking and the origin of sensitivity to chloroethylnitrosoureas, modification of DNA and RNA bases, carbamoylating activity of NUA, chromatin and associated nuclear components as potential drug targets, and ADP-ribosylation and DNA repair as induced by NUA are presented. Clinical studies involve subacute and chronic toxicities associated with NUA therapy, toxicity of high dose carmustine (BCNU) with autologous marrow support, clinical pharmacology of the NUA, NUA in the therapy of Hodgkin's disease, Hodgkin's disease and malignant lymphomas, lomustine (CCNU) in combination chemotherapy programs for small-cell lung cancer, therapy for small-cell anaplastic (oat cell) lung cancer, NUA-containing combinations in small-cell lung cancer, NUA-containing chemotherapy regimens in the treatment of non-small cell lung cancer, chemotherapy of recurrent brain tumors, lipid-soluble NUA in the management of children with primary and recurrent brain tumors, adjuvant therapy of brain tumors, uses of NUA in gastrointestinal cancer, use of NUA in patients with malignant melanoma, multiple myeloma, metastatic sarcomas, high-dose BCNU and autologous bone marrow transplantation, interaction of amphotericin B, in vitro action of NUA in human tumors, streptozotocin, Phase I and II studies of 1-(2-chloroethyl)-3-(2,6-dioxo-1-piperidyl)-1 nitrosourea (PCNU), chlorozotocin, NUA currently under development, and designs for clinical trials. (907 Refs)

50. EFFECT OF CHROMATIN DNASE ON DNA MODIFIED BY THE CARCINOGEN N-ACETOXY-2-ACETYLAMINOFLUORENE.

Vinter VG, Zotkina NL, Khamidullina NG
V. I. Ul'ianov-Lenin State Univ., Kazan, USSR
Eksp Onkol; 4(3):57-59 1982

To evaluate the role of the excision system in liver carcinogenesis, DNA treated with N-acetoxy-2-acetylaminofluorene (AAAF) was incubated with neutral chromatin DNAse. Comparison of the products of hydrolysis of native, AAAF-modified, and denatured DNA showed that the effect of DNAse on the modified DNA was similar to that on the denatured DNA. These findings indicated that neutral chromatin DNAse can recognize and excise the modified bases in native DNA. (15 Refs) (In RUS)

51. ROLE OF ENZYMATIC METHYLATION OF DNA IN CELL DIFFERENTIATION AND TRANSFORMATION.

Fedorov NA
Central Res. Inst. Hematology and Blood Transfusion, Moscow, USSR
Eksp Onkol; 4(3):3-11 1982

Literature data on DNA methylation are reviewed, with special emphasis on the roles of specific DNA methylases and S-adenosylmethionine (SAM) in cell differentiation and neoplastic transformation. Determinations of 5-methylcytosine contents of leukemic and normal lymphocytes showed a marked elevation of DNA methylation in the leukemic cells. Changes in rate and specificity of DNA methylation were observed during chemical carcinogenesis and following administration of antineoplastic agents. A number of SAM analogs were shown to inhibit cell proliferation. (73 Refs) (In RUS)

52. HYPOMETHYLATION OF HELA CELL DNA AND THE ABSENCE OF 5-METHYLCYTOSINE IN SV-40 AND ADENOVIRUS (TYPE 2) DNA: ANALYSIS BY HPLC.

Diala ES, Hoffman RM
Dept. Pediatrics, M-009, Univ. California at San Siego Sch. Medicine, La Jolla, CA, 92093
Biochem Biophys Res Commun; 107(1):19-26 1982

The extent of methylation in highly purified simian virus 40 (SV40) and adenovirus (type 2) DNA was investigated using a sensitive high pressure liquid chromatographic procedure. 5-Methylcytosine was not detected in virion SV40 DNA. This lack of methylation in SV40 contrasts to that in monkey kidney cells in which SV40 is propagated, in which 3.7% of all cytosines are methylated. 5-Methylcytosine was also not detected in adenovirus (type 2) DNA bases. In HeLa cells in which adenovirus is grown, 2.3% of cytosines were found to be methylated. This level of methylation is considerably lower than in normal and SV40-transformed human cells, which is about 3%. The contribution of DNA methylation to SV40 and adenovirus gene activity is discussed. (31 Refs)

53. NON-IMMUNOGLOBULIN-ASSOCIATED DNA REARRANGEMENTS IN MOUSE PLASMACYTOMAS.

Harris LJ, Lang RB, Marcu KB
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Proc Natl Acad Sci USA; 79(13):4175-4179 1982

We have characterized a class of DNA rearrangements in plasmacytomas. These recombination events involve a DNA sequence whose origin is outside of the locus of the heavy chain constant region genes, CH. Therefore, we choose to refer to this sequence as non-immunoglobulin-associated rearranging DNA (NIARD). We have isolated two abortively rearranged C alpha genes, generated by NIARD events from the alpha-producing J558 myeloma. Restriction endonuclease maps of these sequences reveal two possible recombination sites in NIARD that are separated by approx 6.5 kilobase pairs of DNA (defined at 5' and 3' sites). A NIARD rearrangement occurs in 15 out of 20 plasmacytomas tested, including gamma3-, gamma1, gamma2b-, gamma2a-, and alpha-producers, but this event usually does not involve a CH switch (S) region. In fact, only S alpha appears to accept NIARD. How-

ever, NIARD did not undergo a rearrangement in eight IgA-producing hybridomas tested. One germ-line copy of NIARD (a 22-kilobase pair EcoRI fragment) is retained in all plasmacytomas. NIARD does not appear to possess repetitive DNA sequences homologous to S mu or S alpha. We discuss the possible role and implications of NIARD-like sequence rearrangements in allelic exclusion and chromosomal translocation events in plasmacytomas. (Author abstract) (30 Refs)

54. DIFFERENCES IN THE NUCLEASE SENSITIVITY BETWEEN THE TWO ALLELES OF THE IMMUNOGLOBULIN KAPPA LIGHT CHAIN GENES IN MOUSE LIVER AND MYELOMA NUCLEI.

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Nucleic Acids Res; 10(12):3627-3645 1982

In mouse myeloma T the productive kappa light chain gene differs from its aberrantly rearranged allele in the patterns of DNAase I hypersensitive sites. In the region of the alleles where they are identical in sequence they have one site in common which lies 0.8 kb downstream of the coding region; but two sites upstream of and within the C gene segment (2) are found only on the non-productive allele. Within the region of different sequences both alleles have analogously located DNAase I hypersensitive sites; they lie 0.15 kb upstream of the respective leader segments and cover putative promoter sequences. Only one of the six DNAase I hypersensitive sites is also very sensitive towards micrococcal nuclease due to its particular DNA sequence. The non-rearranged gene studied in liver nuclei has no DNAase I hypersensitive sites but is preferentially cleaved in A/T rich regions. (Author abstract) (35 Refs)

55. A SMALL SUBCLASS OF SV40 T ANTIGEN BINDS TO THE VIRAL ORIGIN OF REPLICATION.

Scheller A, Covey L, Barnet B, Prives C
Dept. Biological Sciences, Columbia Univ., New York, NY, 10027
Cell; 29(2):375-383 1982

We examined the affinities of SV40 large T antigen for unique viral DNA sequences by binding SV40 Bst NI DNA fragments in extracts of infected or transformed cells, and then immunoprecipitating the T antigen-DNA complex. The G fragment, which spans the viral origin of replication (ori) was quantitatively bound to T antigen. A T-antigen-specific monoclonal antibody (Mcl 7), which recognized only 5%-10% of the T antigen from infected or transformed cells, immunoprecipitated the majority of the ori-binding activity. This suggests that only a minor subclass of wild-type T antigen is active in binding to the origin. C6 cells contain a replication-defective mutant T antigen that when tested in the DNA-binding immunoassay, showed no affinity for the ori fragment. Mcl 7 not only failed to immunoprecipitate ori binding in C6 cells, but also did not detect any labeled C6 T antigen whatever. Thus Mcl 7 recognizes an immunologically distinct subset of wild-type T antigen that comprises the origin-binding form of the viral protein, which is absent in the C6 T antigen population. Mcl 122, which recognizes a 53 kilodalton host protein that complexes with T antigen, immunoprecipitated ori-binding activity from extracts of infected or transformed cells, but not from C6 cells. Thus wild-type T antigen can bind ori sequences even when complexed to the host protein. These data suggest that T antigen consists of different subpopulations with different functions. (Author abstract) (48 Refs)

56. PROPERTIES OF THE GENOME OF EQUINE HERPESVIRUS TYPE 3.

Atherton SS, Sullivan DC, Dauenhauer SA, Ruyechan WT, O'Callaghan DJ
(c/o O'Callaghan) Dept. Microbiology, Univ. Mississippi Medical Center, Jackson, MS, 39216
Virology; 120(1):18-32 1982

Methods developed for the isolation and purification of equine herpesvirus Type 3 (EHV-3) DNA were shown to yield quantities of intact infectious DNA molecules suitable for characterization by physicochemical, biochemical and electron microscopic methods. Preparations of EHV-3 DNA were shown by CsCl analytical ultracentrifugation to be comprised of a single species of DNA. Rate velocity centrifugation studies revealed that the EHV-3 DNA sedimentation coefficient corresponds to a mol wt value of 90 to 100 megadaltons (Md). Intact viral DNA molecules were found to be 96 to 100 Md by electron microscopy, and restriction enzyme analyses with BamHi, EcoRI, and HindIII yielded an av mol wt of 96.2 Md. Results indicated that a set of DNA sequences located at one terminus is repeated within the genome and that two populations of molecules exist with regard to the orientation of these repeat sequences. Electron microscopic examination of reannealed EHV-3 DNA molecules revealed structures that contained a single-strand loop equivalent to a duplex mol wt of 5.3 Md at one end of the molecule contiguous to a double-strand region which terminated in a long single-strand segment. These structures were identical in morphology to those observed for EHV-1 DNA which has an overall genomic structure of an L (long) region covalently linked to an S (short) region; the S region consists of a unique segment (Us) bracketed by inverted repeat sequences. The above findings indicate that the 96.2-Md EHV-3 genome is comprised of an L region of approx 73-76 Md covalently linked to a 20-to 23-Md S region; the S region contains a 5.3-Md Us segment bounded by 8-Md inverted repeat sequences that allow the S region to invert relative to the fixed L region and the genome to exist in two isomeric arrangements. (57 Refs)

57. MOLECULAR STRUCTURE OF HERPESVIRUS PAPIO DNA AND CLONING OF ITS REPEAT SEQUENCE IN PLASMID PACYC 184.

Lee YS, Nonoyama M, Rabin H
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Excerpta Med Int Congr Ser; 571:12-18 1982

Genetic engineering technology was used to analyze the genomes of herpes virus Papio (HVP) DNA. HVP productive cells (594S/F9) were propagated at 37 C. Virus was isolated from productive cells treated with 12-O-tetradecanoylphorbol-13-acetate. Blotting hybridization and double enzyme digestion was used to form an overall linkage map of HVP DNA. Similarity was found between HVP and Epstein-Barr virus (EBV) in both biological and biochemical aspects. Terminal heterogeneity and internal repeat sequence (IR) were unique features in EBV and HVP DNA and was also found in gorilla DNA isolated from a productive lymphoblastoid cell line. It is suggested that IR might be a unique sequence among all primate EBV-like viruses. (15 Refs)

58. STRUCTURE OF ADENOVIRUS NUCLEOPROTEIN CORE STUDIED BY CIRCULAR DICHROISM AND SELECTIVE RADIOCHEMICAL LABELING.

Boulanger P, Loucheux-Lefevbre MH
Unite de Virologie Moleculaire de l'INSERM, U. 233, Place de Verdun, 59045 Lille Cedex, France
Biochem Biophys Res Commun; 107(2):470-480 1982

The conformational structure of wild-type adenovirus type 2 viral DNA (AV-DNA) within intact virion and adenovirus cores of different protein contents was studied by circular dichroism (CD) and by selective radiochemical labeling of some critical amino acids. Virus was grown on KB cells and was extracted in CsCl gradients. CD spectra were compared in intact virion and in deoxycholate and sarcosyl cores. AD-DNA was in highly condensed and packed conformation in both intact virus particles and deoxycholate cores, as found in chromatin nucleosomes. There were minor but significant CD spectral differences with eukaryotic chromatin which indicated that the adenovirus nucleosome-like structures had specific conformational features. AD-DNA complexed with protein VII in sarcosyl cores elicited a CD spectrum reminiscent of a relaxed DNA, which suggests that the adenovirus sarcosyl cores were no longer organized in nucleosome subunits. In parallel in vitro studies, the nature of the bonds involved in core structure was studied by selective labeling of some critical amino acids. The accessibility of lysyl and tyrosyl residues in adenovirus core proteins was investigated with the use of 14C-dansyl as a probe. Results suggest that core protein V was associated with protein VII mainly via hydrophobic bonds, and had a major role in the maintenance of the nucleosomal structure of the viral core. (34 Refs)

59. THE CHANGES IN PROVIRAL CHROMATIN THAT ACCOMPANY MORPHOLOGICAL VARIATION IN AVIAN SARCOMA VIRUS-INFECTED RAT CELLS.

Chiswell DJ, Gillespie DA, Wyke JA
Amersham International. Amersham, Bucks, England
Nucleic Acids Res; 10(13):3967-3980 1982

The clone All of avian sarcoma virus B77-infected Rat-1 cells comprises both morphologically normal and morphologically transformed derivatives. Transformed subclones, in which virus-specific RNA is readily detectable, contain a provirus that is very sensitive to DNase I digestion of chromatin, and show DNase I hypersensitive sites at the 5' end of the provirus and in 5' flanking cell DNA. Normal subclones with no detectable virus-specific RNA, whether infected cells that have never been transformed or revertants derived from transformed cells, contain a provirus that is far more resistant to DNase I digestion. Moreover this provirus lacks hypersensitive sites at its 5' end, although DNase I hypersensitive sites are present in 5' flanking cell DNA. No DNase I hypersensitive sites were detected at the 3' end of the provirus in either normal or transformed clones. The pattern of cytosine methylation in the proviral restriction sites of the isoschizomers Msp I and Hpa II differed between transformed and revertant clones; the revertants show additional methylation at some CpG doublets. (Author abstract) (42 Refs)

60. DIFFERENTIAL SUSCEPTIBILITY OF HUMAN CELLS TO TRANSFORMATION BY MURINE AND AVIAN SARCOMA VIRUSES.

Rhim JS, Trimmer R, Huebner RJ, Papas Ts, Jay G
NCI, NIH, Bethesda, MD, 20205
Proc Soc Exp Biol Med; 170(3):350-358 1982

The susceptibility of human skin fibroblasts from patients with adenomatosis of the colon and rectum (ACR) and Gardner's syndrome and of human osteosarcoma clonal cells to Kirsten murine sarcoma virus (KiMSV) and Rous sarcoma virus (RSV) was investigated. Results obtained confirmed a previous report that skin fibroblasts from individuals genetically predisposed to colorectal cancer were preferentially transformed by KiMSV. The human osteosarcoma clonal cells infected with RSV showed altered morphology, increased growth rate, higher efficiency of colony formation in soft agar, growth of larger aggregates and growth in aggregate form above an agar base, and tumorigenicity in nude mice. The transformed cells produced neither RNA-dependent DNA polymerase nor type C virus particles; however, the transformed cells contained RSV CF antigen and a rescuable infectious virus. The transformed cells also contained an RSV transformation specific protein p60src-associated kinase activity. (22 Refs)

61. HUMAN DNA SEQUENCE HOMOLOGOUS TO THE TRANSFORMING GENE (MOS) OF MOLONEY MURINE SARCOMA VIRUS.

Watson R, Oskarsson M, Vande Woude GF
Lab. Molecular Oncology, NCI, NIH, Bethesda, MD, 20205
Proc Natl Acad Sci USA; 79(13):4078-4082 1982

We describe the molecular cloning of a 9-kilobase-pair BamHI fragment from human placental DNA containing a sequence homologous to the transforming gene (v-mos) of Moloney murine sarcoma virus. The DNA sequence of the homologous region of human DNA (termed humos) was resolved and compared to that of the mouse cellular homolog of v-mos (termed mumos). The humos gene contained an open reading frame of 346 codons that was aligned with the equivalent mumos DNA sequence by the introduction of two gaps of 15 and 3 bases into the mumos DNA and a single gap of 9 bases into the humos DNA. The aligned coding sequences were 77% homologous and terminated at equivalent opal codons. The humos open reading frame initiated at an ATG found internally in the mumos coding sequence. The polypeptides predicted from the DNA sequence to be encoded by humos and mumos also were found to be extensively homologous, and 253 of 337 amino acids were shared between the two polypeptides. The first five NH2-terminal and last two COOH-terminal amino acids of the humos gene product were in common with those of mumos. In addition, near the middle of the polypeptide chains, four regions ranging from 19 to 26 consecutive amino acids were conserved. However, we have not been able to transform mouse cells with transfected humos DNA fragments or with hybrid DNA recombinants containing humos and retroviral long terminal repeat (LTR) sequences. (Author abstract) (28 Refs)

62. DNA SEQUENCES REQUIRED FOR SPECIFIC AND EFFICIENT INITIATION OF TRANSCRIPTION AT THE POLYOMA VIRUS EARLY PROMOTER.

Jat P, Novak U, Cowie A, Tyndall C, Kamen R
Transcription and Nucleic Acid Chemistry Laboratories, Imperial Cancer Res. Fund, Lincoln's Inn Fields, London WC2A 3PX, England
Mol Cell Biol; 2(7):737-751 1982

The 5'-flanking sequences of the principle polyoma virus (Py) early promoter region was studied by the construction of deletion and substitution mutants. In vivo effects of certain mutations were measured by assay of the ability of DNAs to transform rat fibroblasts; effects of all mutations on the position and efficiency of in vitro initiation were measured with quantitative assays. A convenient internal control was the secondary Py early promoter region. Sequence requirements for efficient in vivo expression were different from those in vitro. DNA deletions between 200 and 400 base pairs (bp) before the principal cap sites severely inhibited in vivo expression measured as transformation ability, while in vitro transcription was not affected. Viable deletion mutants lacking the principal cap sites and the 'TATA' box were poor templates for in vitro transcription. In vitro analysis of other deletion mutants showed that specific sequences more than 46 bp before the cap sites were important. TATA box removal reduced in vitro transcriptional efficiency but did not alter initiation sites. Synthesis of transcripts with abnormal 5' termini did not occur in vitro until the sequence between the TATA box and the normal cap sites was also deleted. There was a nonspecific requirement for 90-100 bp of DNA 5' to the cap site for optimal transcription of DNA fragments in vitro. (73 Refs)

63. CLONING AND STRUCTURAL ANALYSIS OF INTEGRATED WOODCHUCK HEPATITIS VIRUS SEQUENCES FROM HEPATOCELLULAR CARCINOMAS OF WOODCHUCKS.

Ogston CW, Jonak GJ, Rogler CE, Astrin SM, Summers J
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Cell; 29(2):385-394 1982

Woodchuck hepatitis virus (WHV), like the related hepatitis B virus, induces in its natural host hepatocellular carcinomas that contain integrated viral sequences. As a first step in determining whether and how the integrated sequences contribute to formation of the tumors in which they are found, we have cloned two such integrations of WHV and have determined their structure by restriction mapping and heteroduplex electron microscopy. The identity of the cloned sequences was confirmed by comparison of restriction sites in the clones with those located by Southern blot analysis of tumor DNA. Viral sequences in both integrations are extensively rearranged, and in neither were all parts of the viral genome represented. In this respect, the behavior of WHV in vivo is similar to that of other DNA tumor viruses that have been studied in vitro. We discuss the implications of these results in relation to possible mechanisms for tumor induction by WHV. (Author abstract) (38 Refs)

64. A MONOCLONAL IMMUNOBLASTIC SARCOMA IN DONOR CELLS BEARING EPSTEIN-BARR VIRUS GENOMES FOLLOWING ALLOGENEIC MARROW GRAFTING FOR ACUTE LYMPHOBLASTIC LEUKEMIA.

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Blood; 60(1):180-187 1982

A patient undergoing marrow grafting for acute lymphoblastic leukemia from his partially HLA-mismatched sister displayed a widely disseminated immunoblastic sarcoma at autopsy. The tumor was monoclonal by immunoglobulin light-chain staining. Blot hybridization analysis, using a cloned highly polymorphic locus in human DNA as a probe, showed the tumor to be of donor-cell origin. Cytogenetic analysis also demonstrated donor-cell origin. Blot hybridization analysis demonstrated Epstein-Barr virus (EBV) genomes in the tumor. By contrast, reexamination of material from a previously reported case of a donor-type relapse showed no evidence of EBV DNA. In neither case was there evidence of cytomegalovirus DNA. This study documents the association of EBV with a malignant, monoclonal B-cell lymphoma arising in a marrow graft recipient. We conclude that DNA restriction fragment length polymorphisms can be used to prove the origin (donor or host) of neoplastic relapse following allogeneic marrow grafting. Further, cell types different from those of the original leukemia may be involved. (Author abstract) (35 Refs)

65. CHARACTERIZATION OF SV40-TRANSFORMED HUMAN LIVER CELLS BEFORE AND AFTER PASSAGE THROUGH CRISIS.

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Intervirology; 17(4):222-227 1982

SV40-transformed human liver cells could be passed through crisis after transfection with various DNAs (SV40, BKV, or salmon sperm). The pre- and post-crisis cells were compared with respect to growth behavior and the state of the viral genome. Comparison of the viral inserts in the pre- and post-crisis cells showed minor changes in the integration pattern of the SV40 DNA after recovery from crisis. (Author abstract) (18 Refs)

66. HEAT-RESISTANT INFECTIVITY OF HERPES SIMPLEX VIRUS REVEALED BY VIRAL TRANSFECTION.

Fenyves A, Strupp L
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Intervirology; 17(4):228-239 1982

Following apparently effective inactivation of herpes simplex virus Types 1 and 2 at 56 or 70 C, significant infectivity was found upon viral transfection using the calcium-dimethylsulfoxide technique. Infectivity was quantitated in RSB-1 (rabbit skin fibroblast) and BHK-21 cells, and dose-response curves were found to depend on both the cell system and side effects of heat. Titers were dependent upon thermal treatment, as well as initial infectivity and cell origin of virus, and showed no or only moderate decrease after 6-hour exposures. There was no liberation of DNA or DNA- protein complexes, since infectivity was DNase- and trypsin-DNase-resistant. Upon purification,

activity was found associated with particles that had retained their gross physical integrity. Obviously, protein(s) involved in initiation of infection was primarily altered. Heated virus was also insensitive to antiserum, which prevented replication of unheated, transfected virus. (Author abstract) (23 Refs)

67. TRANSFECTION WITH THE ISOLATED HERPES SIMPLEX VIRUS THYMIDINE KINASE GENES. II. EVIDENCE FOR AMPLIFICATION OF VIRAL AND ADJACENT CELLULAR DNA SEQUENCES.

Reyes GR, McLane MW, Hayward GS
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J Gen Virol; 60(part2):209-224 1982

We have previously described several series of biochemical transformation experiments in which small defined portions of herpes simplex virus (HSV-1 and HSV-2) DNA encompassing the thymidine kinase (TK) gene were introduced into Ltk- cells by the calcium transfection procedure. The presence of authentic virus TK enzyme in several subcloned cell lines derived from these experiments was confirmed by either the specific incorporation of [125I]iododeoxycytidine into their nuclei or the inhibition of cell growth by the antiviral drug arabinosyl thymine. A panel of 24 independent Ltk+ cell lines receiving either isolated virus DNA fragments or cleaved plasmid DNAs was examined by blot hybridization for both the presence and copy number of virus TK DNA sequences. Most cell lines contained a single virus DNA fragment covalently joined to host (or carrier Ltk(-)) mouse DNA sequences, but several contained multiple copies of the TK gene. Examination of the structural arrangement of the virus DNA in two early passage multicopy cell lines indicated that the TK gene had integrated into Ltk- cell DNA and then subsequently both viral and flanking cellular sequences were amplified to create up to 20 tandem duplications. In one case, mapping of the adjacent cellular sequences has revealed that the total repeat unit is greater than 23 kilobases (kb) in size. On subsequent passaging, even in HAT medium, the amplified repeat units were not stable and fell to only three to four copies per haploid cell genome. These cell lines should prove useful for additional studies to examine the expression of co-selected non-TK virus sequences and the influence of adjacent cellular DNA sequences on transcription and retransfection of the resident TK gene. (Author abstract) (45 Refs)

68. A NOVEL CELL TRANSFORMATION WITH DNA-CONTAINING CYTOPLASMIC YABA TUMOR POXVIRUS.

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Lab. Molecular and Cancer Virology, Dept. Microbiology, Southern Illinois Univ., Carbondale, IL, 62901
Virology; 120(1):77-92 1982

Monkey kidney cells were morphologically transformed in vitro with uv-irradiated Yaba tumor poxvirus. Cell lines established were shown to be virus nonproducers and exhibited biological characteristics typical of transformed cells. These characteristics included increased saturation density, reduced serum requirements for growth, and ability to grow in soft agar. The morphological alterations of transformed cells were similar to Yaba virus-induced tumor cells and were characterized by loss of contact inhibition, multinucleated cells, and cytoplasmic lipid droplets. Southern blot hybridization revealed that sequences homologous to low-mol-wt viral DNA were present in the transformed cells. Yaba virus-specific antigens detected by immunofluorescence assays were found to be in the cytoplasm of transformed cells. Four virus-specific proteins, with mol wt of 160,000, 140,000 107,000, and 74,000 daltons, were detected in transformed cells immunoprecipitated with sera from tumor-bearing monkeys. These results indicate that DNA-containing Yaba tumor virus, which replicates exclusively in the cytoplasm, is capable of inducing cell transformation in vitro. (Author abstract) (32 Refs)

69. DELETION MUTANTS WHICH AFFECT THE NUCLEASE-SENSITIVE SITE IN SIMIAN VIRUS 40 CHROMATIN.

Gerard RD, Woodworth-Gutai M, Scott WA
Dept. Biochemistry, Univ. Miami Sch. Medicine, Miami, FL, 33101
Mol Cell Biol; 2(7):782-788 1982

Nuclear extracts from in(Or)-1411-infected cells were incubated with MnCl2, allowing endogenous nuclease to cleave within the nuclease-sensitive regions. Introduction of deletions into a simian virus 40 (SV40) variant enabled a quantitative comparison between the nuclease sensitivity of the segment carrying the deletion and the unaltered copy elsewhere on the viral genome. Restriction enzyme analysis and heteroduplex mapping gave patterns identical to that of in(Or)-1411 DNA except for deletion of sequences within the inserted segment. Endonuclease which is endogenous to BSC-1 cell nuclei was used to cleave 32P-labeled chromatin to a limited extent. Variant in(Or)-1411 preferentially cut within each of the duplicate segments. Deletion mutant chromatin displayed nuclease sensitivity in segment I which is comparable to the parent. In(Or)-1411-dl12, -dl14, and -dl46 showed little or no preferential cleavage in region II. Mutants dl9, dl10, dl16, and dl42 retained the characteristic nuclease sensitivity in region II. Locations of the preferentially nuclease-sensitive regions were confirmed by restriction enzyme mapping of the linear DNA with KpnI. Specific cutting frequencies (corrected for a background assumed to be due to random cleavage) obtained for segments I and II in the mutants are presented. (29 Refs)

70. THE CHARACTERIZATION OF NON-HISTONE PROTEINS WHOSE AMOUNTS INCREASE IN CHROMATIN FROM MOUSE HEPATOCARCINOMAS.

Medvedev ZA, Buchanan JH, Medvedeva MN, Crowne HM
Div Genetics, Natl. Inst. Medical Res., Mill Hill, London NW7 1AA, England
Int J Cancer; 30(1):87-92 1982

A fraction containing liver- and hepatoma-specific non-histone proteins has been isolated from the chromatin of mice. Amino acid analysis of this fraction shows that it contains 16 mol of glutamic acid, 10 mol aspartic acid, 7 mol of both arginine and lysine per 100 mol and contains no cysteine or tyrosine. The proteins in this fraction are strongly associated with DNA and are co-extracted with histones from chromatin with 0.25 M HCl. In chromatin from age-related hepatomas, the amount of this fraction increased six-fold. This increase in concentration of these chromatin proteins may be associated with changes of chromatin structure necessary to initiate malignant growth in liver cells. (Author abstract) (33 Refs)

OTHER RELATED STUDIES

71. SEPARATION AND CHARACTERIZATION OF NEOPLASTIC CELL SUBPOPULATIONS OF A TRANSPLANTABLE RAT PANCREATIC ACINAR CARCINOMA.

Becich MJ, Reddy JK
(c/o Reddy) Dept. Pathology, Northwestern Univ. Medical Sch., 303 East Chicago Ave., Chicago, IL, 60611
Cancer Res; 42(9):3729-3740 1982

The transplantable pancreatic acinar carcinoma established in F344 rats demonstrates heterogeneity of cytodifferentiation with cell types ranging from those containing no zymogen granules to cells with mature zymogen differentiation. Two relatively homogeneous subpopulations of cells were isolated by isopyknic Percoll gradient centrifugation from the heterogeneous cell population of this tumor. The subpopulation obtained at a density of 1.0987 g/ml was designated the granule-enriched fraction (GEF) and contained morphologically differentiated cells with abundant mature zymogen granules. The other subpopulation, obtained at a density of 1.0789 g/ml, consisted of poorly differentiated cells lacking zymogen maturation and was thus termed the granule-deficient fraction (GDF). In both fractions, greater than 97% of the cells were viable and maintained linear rates of [3H]leucine and [3H]thymidine incorporation into protein and DNA for up to 3 hr following isolation. Twenty percent of the cells in GDF subpopulation and 12% of the cells in GEF subpopulation synthesize DNA. Distinct differences in nuclear:cytoplasmic ratio, secretory granule content, and degree of polarity were found between GDF and GEF. The GEF subpopulation contained high levels of amylase and lipase, while the GDF subpopulation displayed very low levels of these enzymes. Both subpopulations produced tumors when injected sc into F344 rats. Concanavalin A (Con A) receptors were found on pancreatic carcinoma cells regardless of the extent of cytodifferentiation in contrast to normal pancreatic embryogenesis, in which Con A receptors are discerned only in acinar cells containing mature secretory granules. This finding suggests either that the neoplastic transformation results in altered genetic expression or that pancreatic carcinoma arises from dedifferentiation of mature acinar cells which normally possess Con A receptors. (52 Refs)

72. CURRENT APPROACHES TO THE EXPERIMENTAL INVESTIGATION OF CHEMICALS IN RELATION TO CANCER OF THE BRAIN.

Swenberg JA, Zimmerman HM, Maltoni C, Kurland L, Laerum OD, Kleihues P, Selikoff I, Heidelberger C
Chemical Industry Inst. Toxicology, Research Triangle Park, NC, 27709
Ann NY Acad Sci; 381:43-53 1982

Chemical induction of brain tumors, current methods for evaluating the carcinogenic potential of chemicals, and possible mechanisms of neuro-oncogenesis are reviewed. Chemical induction of brain tumors by polycyclic aromatic hydrocarbons, nitroso compounds, acrylonitrile, and other chemicals (1-aryl-3,3-dialkyltriazenes, 1,2-diethylhydrazine, azoethane, azoxyethane, and procarbazine) is described. Only a small number of chemicals that induce brain tumors have been identified by routine bioassays of new chemicals. Current guidelines require exposure of a minimum of 50 animals of each sex to 2-3 exposure levels and an equal number of controls. Complete necropsies, diagnosis by an experienced histopathologist, and confirmatory histochemical techniques are essential for properly classified brain tumors. Knowledge of normal variation in tumor incidence between species, strain, and sex is required. A positive bioassay should induce a significantly increased incidence of tumors in a dose-related manner. Possible mechanisms involved in neuro-oncogenesis have been proposed, including the formation and persistance of O6-alkylguanine in brain DNA of rodents and the occurrence of glial cell replication in the presence of promutagenic DNA damage. (48 Refs)

73. ACTIONS OF CIS-DIAMMINEDICHLOROPLATINUM ON CELL SURFACE NUCLEIC ACIDS IN CANCER CELLS AS DETERMINED BY CELL ELECTROPHORESIS TECHNIQUES.

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Cancer Res; 42(9):3565-3573 1982

Whole-cell electrophoresis determinations were made on a variety of tumor cell suspensions in order to examine the charged groups on the cell surfaces. We report here evidence which indicates the presence of nucleic acids on the surfaces of the tumor cells. Cells of the ascitic and solid forms of the Sarcoma 180 mouse tumor, grown in ICR mice, showed a decrease of 10 to 20% in electrophoretic mobility when incubated with nucleases. One variant, S-180, exhibited sensitivity to RNase only; another variant was sensitive to both RNase and DNase, while different tumor lines were shown to be sensitive to DNase only. Treatment of tumor-bearing animals with a therapeutic dose of cisplatin resulted in a loss of tumor cell mobility identical to that produced by the nuclease incubations of the control cells. Incubation of the cisplatin-treated tumor cells with nucleases produced no change in mobility. The isoelectric points of these cells are consistent with the loss of a group with a low pK value in both cisplatin-treated cells and nuclease-incubated cells. Using the S-180 tumor sensitive to both RNase and DNase, the rates of the two enzymes were additive, but the mobility decreased to the same level regardless of whether the enzymes were used alone or together. This suggests that both enzymes act on the same site. RNase and DNase immobilized on agarose beads were capable of lowering the mobility of the cells upon incubation, confirming the surface location of these nucleic acid residues. S-180 tumor cells were also placed in a tissue culture medium at 37 C for up to 22 hr and were treated in vitro with cisplatin and other metabolic inhibitors. Nucleic acid or protein synthesis inhibitors produced a loss of the cell surface nucleic acids. In another in vitro experiment, the nucleic acids were first removed by nucleases, and, when the cells were incubated at 37 C, the nucleic acids reappeared. Disappearance with the inhibitors or reappearance after digestion exhibited a half-time of about 2 hr. Surface nucleic acids were detected by this electrophoresis technique on all of several types of tumor cells but not on any normal cells examined. (18 Refs)

74. EFFECT OF ACYCLOVIR ON THE DEOXYRIBONUCLEOSIDE TRIPHOSPHATE POOL LEVELS IN VERO CELLS INFECTED WITH HERPES SIMPLEX VIRUS TYPE 1.

Furman PA, Lambe CU, Nelson DJ
Dept. Experimental Therapy, Wellcome Res. Lab., Research Triangle Park, NC, 27709
Am J Med; 73(1A):14-17 1982

The effect of acyclovir on the deoxyribonucleoside triphosphate pools of Vero cells infected with herpes simplex virus Type 1 was examined. Deoxyguanosine triphosphate and deox-

yadenosine triphosphate pool levels in infected cells treated with acyclovir increased dramatically compared with pool levels in untreated infected cells. The increases were due, at least in part, to inhibition of viral DNA polymerase activity which resulted in reduced utilization of the deoxyribonucleoside triphosphates. Differences of as much as 26x were detected in the sensitivity of herpes simplex virus Type 1 to inhibition by acyclovir with different Vero cell cultures. These results were due to differences in acyclovir triphosphate levels, not to differences in deoxyguanosine triphosphate levels. (Author abstract) (18 Refs)

75. A CONTRIBUTION TO THE INVESTIGATION OF TARGET CELLS IN N-NITROSO-N-METHYL UREA-INDUCED LEUKEMOGENESIS IN MICE.

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Arch Geschwulstforsch; 52(6):451-458 1982

Chemical leukemogenesis by administration of N-nitroso-N-methyl urea (NMU) was studied in (XVII x AKR)F1 rats to determine the target organ and target cells of the carcinogen. NMU was labeled with NMU (0.1 mg/g body wt) was injected ip or sc into rats of various ages. NMU was administered in a single dose or in two fractions at an interval of 4 wk. Rats were killed at 5 hr, 1, 2, 4, and 15 days after injection of the labeled NMU. Blood, lungs, liver, spleen, kidney, lymph nodes, thymus, bone marrow, and brain tissue were examined for MNU content by scintillation counting and autoradiography. The DNA content of thymus, bone marrow, lymph nodes, and spleen ranged from 8,200 to 9700 ug/g tissue. Labeled NMU reached the highest concentrations in the bone marrow. Significant amounts (5,500 disintegrations/mg: dpm/mg bone marrow) of NMU were found 5 hr after injection. The lowest amounts were found in the blood and brain (150 dpm/mg). Values for lung, liver, spleen, kidney, lymph node, and thymus tissue were 300-1,200 dpm/mg. Since DNA synthesis was approx equal in bone marrow, thymus, and lymph node tissue, the increased labeling of bone marrow with NMU was specific for bone marrow. The bone marrow is evidently the target organ for leukemogenesis by NMU. Lymphatic cells in the marrow are probably the target cells. The thymus apparently is not essential for lymphatic leukemogenesis. (37 Refs) (In GER)

76. DNA DETERMINATION IN THE CYTOLOGICAL DIAGNOSIS OF THYROID TUMORS WITH FOLLICULAR STRUCTURE.

Lukacs G, Balazs Gy, Zs-Nagy I, Csaky G, Fabrian E
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Magy Onkol; 26(2):197-205 1982

Cytofluorometric determination of the DNA content in cell nuclei and cytochemical tests were performed in fine-needle biopsy specimens obtained from 75 patients with suspected malignant thyroid tumors. Strongly increased DNA levels were found in the cell nuclei in undifferentiated thyroid tumors in agreement with the high proliferation rate of these tumors. The increase was smaller in medullary tumors. The investigation of the nuclear DNA levels in tumors with follicular structure revealed a shift from diploid toward tetraploid DNA levels, indicative of the presence of two different cell populations, in the patients with follicular adenomas. The DNA levels in follicular carcinomas were found to range from diploid to octoploid (2c-8c), with 10.3% of the cells being beyond 8c. These results show pronounced aneuploidy in follicular carcinoma. Examinations of the nuclear DNA levels in multiple nodules from the same patient revealed significantly higher DNA levels in follicular adenomatous components than in follicular or medullary carcinomatous components. The findings indicate the value of DNA cytofluorometry in the differential diagnosis of thyroid adenomas and carcinomas with follicular structures. (25 Refs) (In HUN)

77. EFFECT OF ENZYMATIC DISAGGREGATIN ON PROLIFERATION OF HUMAN TUMOR CELLS IN SOFT AGAR.

Hamburger AW, White CP, Tencer K
Cell Culture Dept., American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, 20852
JNCI; 68(6):945-949 1982

Cell suspensions were prepared by either mechanical or enzymatic disaggregation methods from biopsy specimens from 54 patients with various tumors. The biologic activities of cells derived from the two suspensions were then examined. Biopsy specimens of solid tumors were minced, and one-half of each specimen was further processed by being teased with needles, whereas the other half was exposed to a combination of collagenase, hyaluronidase, and DNase. The enzymatic disaggregation method yielded fewer cells per gram of tissue than the mechanical method. However, the percentage of dye-excluding cells was increased by the enzymatic procedure in 93% of the cases. Cells obtained by enzymatic means also had higher cloning efficiencies than those obtained by mincing. The histologic types of cells present in the initial cell suspensions were the same for cells obtained by the enzymatic or mechanical disaggregation methods. The number of colonies obtained was linearly related to the number of cells plated in both cases. The tritiated thymidine sucide indices (estimates of the percentage of cells in the S-phase of the cell cycle) were the same for the two cell populations obtained by the two methods. The results indicate that cells obtained from solid tumors by enzymatic dissociation methods did not differ significantly from cells obtained by the more conventional mechanical techniques. However, cell viabilities and cloning efficiencies were significantly improved by the enzymatic technique. (Author abstract) (22 Refs)

78. CHARACTERISTICS OF SEVEN CLONES OF THE WIDR HUMAN COLON ADENOCARCINOMA CELL LINE.

Petricciani JC, Smith P, Earley EM, Levenbook I, Noguchi P
Bureau Biologics, Food and Drug Admin., 8800 Rockville Pike, Bethesda, MD, 20205
In Vitro; 18(5):492-494 1982

Seven clonal populations were derived from the WiDr human colon adenocarcinoma cell line and were characterized with respect to chromosome number. DNA content, and tumorigenicity in nude mice. There was no correlation between tumor vol and either DNA content or chromosome number; but there were wide differences among the clones regarding the size of tumors they were able to produce in nude mice. (author abstract) (8 Refs)

79. ESTABLISHMENT OF VINCRISTINE-RESISTANT AND VINDESINE-RESISTANT LINES OF MURINE LYMPHOBLASTS IN VITRO AND CHARACTERIZATION OF THEIR PATTERNS OF CROSS-RESISTANCE AND DRUG SENSITIVITIES.

Hill BT, Whelan RD
Lab. Cellular Chemotherapy, Imperial Cancer Res. Fund, Lincoln's Inn Fields, London WC2A 3PX, England
Cancer Chemother Pharmacol; 8(2):163-169 1982

The possible lack of cross responsiveness between vincristine (VCR) and vindesine (VDS) was investigated utilizing newly developed drug-resistant cell lines from L5178Y lymphoblasts. Cell lines resistant to either drug were easily established following a limited exposure to high drug concentrations of 200 nanog/ml. Resistant lines were similar to parental cells in terms of doubling time, cell cycle distribution, cell volume, modal chromosome number, and cellular DNA, RNA and protein contents. Cross resistance between VCR and VDS was not invariable and appeared to be dose dependent. Either partial or complete resistance was observed to other vinca alkaloids, to the anthracyclines, and to actinomycin D (ACT D). Full sensitivity was retained to methotrexate and 5-fluorouracil. Both the VCR-resistant and VDS-resistant lines exhibited comparable patterns of sensitivity or resistance to other antitumor drugs. The VDS-resistant line exhibited cross resistance to both ACT D and 4'-(9-acridinylamino)- methanesulfon-m-anisidine (mAMSA), while the VCR-resistant line showed only slight resistance to ACT D but full sensitivity to mAMSA. This suggests that mAMSA may be valuable in specifically overcoming resistance to VCR, suggesting that VDS may be valuable as second-line therapy in tumors equally responsive to the two vinca alkaloids. (32 Refs)

80. HYBRIDS BETWEEN MYELOMA CELLS AND THYMOCYTES. AN ATTEMPT TO APPROACH THE BIOLOGICAL FUNCTION OF TERMINAL TRANSFERASE.

Transy C, Rouget P
(c/o Rouget) Institut de Recherche en Biologie Moleculaire C.N.R.S., Universite Paris 7, 2, place Jussieu, 75251 Paris Cedex 05, France
Adv Exp Med Biol; 145:207-219 1982

Biological function of terminal deoxynucleotidyl transferase (TdT) as a possible generator of immunological diversity was studied using thymocytes and pre-T lines as parental TdT-positive cells. Hybrids between thymocytes an B cells arose with a frequency somewhat similar to that observed for homologous hybrids. Most of the obtained hybrids presented and secreted surface immunoglobulins which had conserved all the tested characteristics of the ABPC-48 parental Ig: anti-levan activity, and allotypic and idiotypic determinants. (34 Refs)

81. DIFFERENTIAL CELL CYCLE PHASE SPECIFICITY FOR NEOPLASTIC TRANSFORMATION AND MUTATION TO OUABAIN RESISTANCE INDUCED BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE IS SYNCHRONIZED C3H10T1/2 CL8 CELLS.

McCormick PJ, Bertram JS
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Proc Natl Acad Sci USA; 79(14,part1):4342-4346 1982

The transformable mouse embryo fibroblast cell line C3H10T1/2 Cl8 has been employed to study the induction by the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) of morphological transformation and mutation to ouabain resistance throughout the cell cycle. Cells were synchronized by means of isoleucine deprivation for 24 hr and initiated DNA synthesis with a high degree of synchrony 7.5 hr after release of the isoleucine block. At various intervals throughout the cell cycle cultures were treated with MNNG at 1.0 ug/ml and the induction of cytotoxicity, morphological transformation, and ouabain-resistant colonies was determined. All three phenomena exhibited marked cell-cycle phase dependency. Maximal induction of transformation occurred in cultures treated 7.5 hr after release from isoleucine deprivation, when the cells were at the G1/S boundary. In contrast, induction of ouabain-resistant colonies was at a minimum at the time of maximal induction of transformation, and peak induction of ouabain resistance did not occur until 16-18 hr after release from the isoleucine block, when cells were in late S phase. A close correlation was observed between the induction of cytotoxicity and of ouabain-resistant mutants. The results suggest that differences exist in the production or cellular processing of the various early lesions. (Author abstract) (41 Refs)

82. PROLIFERATION KINETICS AND SPECIFIC BEHAVIOUR OF G2 CELLS OF THE BP8 MOUSE ASCITES SARCOMA IN VIVO.

Cao S, Skog S, Brahme A, Tribukait B
Dept. Medical Radiation Biology, Karolinska Institutet, S-1041 Stockholm, Sweden
Acta Radiol Oncol Radiat Phys Biol; 21(1):17-25 1982

Growth characteristics of the Bp8 ascites sarcoma were investigated by means of evaluation of total cell number, the cellular DNA content, the mitotic index and the percentage of dead cells. Following intraperitoneal inoculation of 18 x 10(6) cells into NMRI mice a plateau level of about 1,400 x 10(6) cells was reached after 7 days followed by a daily decline of 5% up to Day 14, the terminal stage of life. Based on the increase of the cell number and the distribution of cells in the cell cycle the flow rate through and the duration of the various parts of the cell cycle were calculated. Generally, the durations of all phases of the cell cycle including mitosis increased with age, most markedly after Day 8 when the durations tended to become infinite. At the plateau phase the total number of G1, S-phase and mitotic cells decreased significantly while G2 cells remained at a constant level. This finding may be of interest in view of the concept of ascites tumor cells behaving as a regulated cell system. (Author abstract) (14 Refs)

83. STABILITY AND COMPONENTS OF MATURE SIMIAN VIRUS 40.

Bina M, Beecher S, Blasquez V
Dept. Chemistry, Div. Biochemistry, Purdue Univ., West Lafayette, IN, 47907
Biochemistry; 21(13):3057-3063 1982

We have examined the stability of mature simian virus 40 (SV40) to changes in pH and have investigated the DNA-specific enzymatic activities that are released from SV40 virions. Our studies show that when SV40 virions are disrupted by the alkaline disruption method described by Brady et al, In addition to the capsid proteins, a DNA topoisomerase and a DNA endonuclease are released. Treatment with a reducing agent and divalent ion chelator is required for the release of both enzymes from the mature virions. Kasamatsu and Wu have previously isolated from SV40 virions a protein covalently linked to SV40 DNA under strongly denaturing conditions. Considering the known biochemical properties of DNA topoisomerases, we propose that the protein in Kasamatsu and Wu's isolated pro-

tein-DNA complex is the SV40-associated topoisomerase. In this work, we show that the topoisomerase is activated by Mg++ ions and does not require a high-energy cofactor to relax supercoiled DNA. On the other hand, the endonuclease is active at 37 C only in the presence of Mg++ ions. While the topoisomerase and the endonuclease activities are released from the virions near neutral conditions, the extent of dissociation of capsid proteins from SV40 chromosomes and the shape of subviral DNA-protein complexes depend on pH. At pH 10.4, the DNA-containing particles sediment at 60 S. At lower pH values, 9.8 and 9.2, the subviral nucleoprotein complexes sediment at 75 and 92 S, respectively. Below pH 9.0, the virus particles decay to a heterogeneous mixture of DNA-containing particles. The majority of the nucleoprotein complexes that result from disruption of the virions at pH 8.8 appears as highly compact SV40 chromatin. Electron microscopic observations and biochemical analysis suggest that a fraction of encapsidated SV40 chromatin seems to be deficient in histones or 'normal' nucleosomes. (Author abstract) (39 Refs)

84. SELECTIVE INHIBITION OF ADENOVIRUS TYPE 2 EARLY REGION II AND III TRANSCRIPTION BY AN ANISOMYCIN BLOCK OF PROTEIN SYNTHESIS.

Shaw AR, Ziff EB
Rockfeller Univ., New York, NY, 10021
Mol Cell Biol; 2(7):789-799 1982

Transcriptional regulation of adenovirus Type 2 (Ad-2) early gene expression was studied. The early period was dissected into a series of stages by blocking protein synthesis with the antibiotic anisomycin. Selective blockage of EII and EIII was used to assess the role of chromatin structure change in the activities on EII and EIII. Anisomycin (100 uM) was added to one portion of an exponentially growing HeLa cell culture, and 30 min later the culture and drug-free control were concentrated for virus infection. In the control culture, EIa, EII, EIII, and EIV were transcriptionally active, and nuclear RNA from each of the regions accumulated. In the drug-treated culture, EIa and EIV were also active, and RNA accumulation was comparable to that of the control. Accumulation of messenger RNA (mRNA) from regions EIa and EIV proceeded at essentially the same rate in the presence or absence of the drug. Anisomycin treatment caused a dramatic decrease, estimated to be greater than 10-fold, in the steady-state level of EII and EIII mRNA. The EII-EIII transcription rate was greatly repressed in the presence of anisomycin. Results confirmed that the principal effect of anisomycin is at the level of transcription and is confined to regions EII and EIII. Similarity in the banding patterns suggested that a protein synthesis block which decreases transcription from the EII and EIII promoters by greater than 90% does not cause a difference in the pattern of deoxyribonuclease I sensitivity of the repressed chromatin relative to the transcriptionally active chromatin in control cells. (26 Refs)

85. ANALYSIS OF YABA TUMOR POXVIRUS-INDUCED PROTEINS IN INFECTED CELLS BY TWO-DIMENSIONAL GEL ELECTROPHORESIS.

Vafai A, Rouhandeh H
(c/o Rouhandeh) Lab. Molecular and Cancer Virology, Dept. Microbiology, Southern Illinois Univ. at Carbondale, Carbondale, IL, 62901
Virology; 120(1):65-76 1982

Lack of inhibition of host protein synthesis by Yaba tumor poxvirus and comigration of viral and host proteins did not interfere with high resolution of virus-induced proteins when two-dimensional gel electrophoresis was employed. At total of 84 virus-induced proteins, 44 structural and 40 nonstructural, comprising 62.9% of the coding capacity of Yaba virus DNA were detected. These proteins may be further categorized in two classes: early, synthesized in the presence of DNA inhibitor, Ara-c; and late, beginning at 6 hr pi with the bulk of viral protein synthesis occurring later than 30 hr pi The rate of synthesis of viral proteins fell into two patterns: Group A, in which the rate of synthesis decreased; and, Group B, in which the rate of synthesis increased after the initial appearance of the protein. (Author abstract) (35 Refs)

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